# THE ELECTROCHEMISTY OF DENTAL ENAMEL AND CARIES

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# DE ELEKTROCHEMIE VAN TANDGLAZUUR EN KARIES

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*aan mijn ouders,*
*aan Mieke, Martijn en Selma*
*in nagedachtenis aan Femke*

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C O N T E N T S.

# 1 INTRODUCTION

## *1.1 Some Properties of Dental Enamel.*

Dental enamel is a hard substance, built up of tiny crystals of one or more poorly soluble calcium phosphates. These crystallites, which have a cross section of about 35 x 90 nm and are from 200 to 1000 nm long (Frank, 1973), are embedded in a matrix which mainly consists of lipis, proteins and water. The volume percentages of the calcium phosphates, the organic compounds and water are on the average 87, 3 and 10 respectively.

The calcium phosphate which forms the major part of enamel has a structure and composition related to that of hydroxylapatite, $Ca_{10}(PO_4)_6(OH)_2$. It is, however, in no way a pure hydroxylapatite, as all ions in the crystallatice can be replaced partially by a number of others. The most common substitutions, either isomorphous or metamorphic, for $Ca^{2+}$ are: $Na^+$, $Mg^{++}$, $K^+$, $Sr^{++}$ and $Pb^{++}$. The most common substitutions for $PO_4^{\equiv}$ are: $HPO_4^{=}$ and $CO_3^{=}$, and $OH^-$ can be substituted by $CO_3^{=}$, $F^-$ or $Cl^-$. A relatively large number of $Ca^{++}$ and $OH^-$ sites can be vacant. A formula for a biological apatite, including the most common substitutions as occurring in human enamel, could be (Driessens, 1973):

$$Ca_9Na_{0.25}Mg_{0.15}K_{0.04}{}^{V(Ca)}{}_{0.56}\{(PO_4)_{5.42}(HPO_4)_{0.08}(CO_3)_{0.5}\}$$
$$OH(CO_3)_{0.05}Cl_{0.06}F_{0.005}{}^{V(OH)}{}_{0.88} \qquad 1.1.1$$

Part of the minority ions, however, might be accounted for by adsorption onto the surface (Driessens, 1973). In the next chapters we shall refer to such a biological apatite simply as "apatite" and when some specific apatite is meant it will be called by its proper name, like hydroxylapatite.

One of the most important consequences of enamel being

built up of crystallites in a gel matrix is that it is porous. The pores of dental enamel are sufficiently wide to allow small organic and inorganic ions and molecules to diffuse through it. Zahradnik and Moreno (1975) have calculated the size distribution of these pores from water vapor sorption isotherms. They found a distribution for the radii of the pores with two maxima, one around 1.0 nm, the other around 2.5 nm. Borggreven, van Dijk and Driessens (1976) have measured the diffusion coefficients of sorbitol, glycerol, $Rb^+$ and chloride. They found values ranging from $10^{-7}$ to $10^{-9}$ $cm^2$ $s^{-1}$ for different specimens of bovine enamel. Burke and Moreno (1975) have determined the diffusion coefficient for tritiated water and found $10^{-8}$ to $10^{-9}$ $cm^2$ $s^{-1}$ for human enamel. Although these values are a few orders of magnitude smaller than those in bulk water, diffusion through dental enamel can be important in the caries process.

The diffusion of molecules through dental enamel is a function of the gradient of their chemical potential and their diffusion coefficients. The diffusion coefficient is a function of the porosity of enamel and the size and shape of the hydrated molecule. If the porosity is decreased the diffusion of molecules will decrease too. The diffusion of ions is more complicated as, in addition to a chemical potential an electrical potential is of importance. An electrical potential will develop if the enamel is more permeable for either cations or anions, or in other words if it is ionselective. Klein, Williams and Amberson (1926) and Waters (1971) proved that such electrical potentials develop across enamel. Enamel appeared to be cation-selective in most cases.

As the total flux of electrical charge through dental enamel should be zero, decreasing the flux for one type of ion will

also decreases the flux of the other type. Thus, both a decrease in porosity and an increase of ionselectively will decrease the flux of ions through the enamel.

*1.2 The Solubility of Tooth Enamel.*

Apatite is the calcium salt of a weak acid. This means that the solubility is not a simple function of the composition of the solution as in the case of a poorly soluble salt of a strong acid like AgCl. If hydroxylapatite (OHA) dissolves $Ca^{2+}$, $PO_4^{3-}$ and $OH^-$ ions come into solution. The $OH^-$ and $PO_4^{3-}$ ions will instantaneously react with $H^+$ ions from the solution until equilibrium is attained. The calcium ions will react with various phosphate ions to form soluble calcium phosphate complexes. In the case of pure OHA and a solution that contains only calcium and phosphate ions, the following equilibria play a role (Moreno, 1968).

$$Ca_{10}(PO_4)_6(OH)_2 \rightleftarrows 10\ Ca^{2+} + 6\ PO_4^{3-} + 2\ OH^- \qquad K_A$$

$$OH^- + H^+ \rightleftarrows H_2O \qquad K_w$$

$$PO_4^{3-} + H^+ \rightleftarrows H\ PO_4^{2-} \qquad K_3$$

$$H\ PO_4^{2-} + H^+ \rightleftarrows H_2PO_4^- \qquad K_2$$

$$H_2PO_4^- + H^+ \rightleftarrows H_3PO_4 \qquad K_1$$

$$Ca^{2+} + H_2PO_4^- \rightleftarrows Ca\ H_2PO_4^+ \qquad K_5$$

$$Ca^{2+} + H\ PO_4^{2-} \rightleftarrows Ca\ H\ PO_4^o \qquad K_6$$

If we assume the concentrations of $H_2PO_4^-$ and $HPO_4^{2-}$ to be large as compared to the other phosphate containing compounds in the solution and the concentration of $Ca^{++}$ large as compared to the calcium containing complexes and if the activities

are assumed to be equal to the concentrations, the following formula can be derived:

$$[Ca_{tot}] = K_A^{0.1} \; [H^+]^{0.2} \left\{ \frac{[H^+] \; ([H^+] + K_2)}{[P_{tot}]} \right\}^{0.6} \frac{1}{(K_2 K_3)^{0.6} \; K_w^{0.2}} \qquad 1.2.1$$

A good approximation to this formula between pH = 5 and pH = 7 has the following form:

$$[Ca_{tot}] = K_A^{0.1} \; \frac{[H^+]^{1.34}}{[P_{tot}]^{0.6}} \; . \; 2.55 \; 10^{14} \text{ mol } l^{-1} \qquad 1.2.3$$

If the total calcium and phosphate concentrations have the same ratio in solution as in the solid phase this expression becomes

$$[Ca_{tot}] = K_A^{0.0625} \; [H^+]^{0.839} \; 1.22 \; 10^9 \text{ mol } l^{-1} \qquad 1.2.4$$

One should realize that at lower pH values the concentrations of phosphate and calcium become quite high and that thus the activities of especially the polyvalent ions become considerably lower than the concentrations. The formulas, however, present good starting values for more precise calculations and show how the solubility of OHA is a function of the pH of the solution. It shows that at pH = 5 the solubility is at least 50 times the solubility at pH = 7 if there is no excess calcium or phosphate in the solution.

The fact that various ions can be built in the apatite lattice makes the solubility behaviour of apatite still more complicated. The incorporation of fluoride instead of hydroxyl lowers the solubility product. The incorporation of carbonate, which is quite abundant in the human body, seems to make the

solubility product higher (Driessens, Borggreven and Van Dijk, 1977). In contact with a solution, the composition of the outer surface of the apatite crystals will, by exchange of ions, shift in such a direction that it becomes more in equilibrium with the solution, changing the apparent solubility product of the apatite. Thus the apparent solubility product of an apatite not only depends on its bulk composition but also on the composition of the solutions with which it has been in contact. Patel and Brown (1975) measured the solubility product of powdered, human dental enamel as a function of the total amount of calcium dissolved. They found that with an increasing amount of calcium dissolved the solubility product decreases from $1.4\ 10^{-105}$ to $1.3\ 10^{-115}$ . This means that the solubility of enamel at a certain pH in the range of 5 to 7 can vary with a factor four (formula 1.2.4). This property of enamel will affect the results of almost all experiments done with it. In our experiments we tried to use solutions which were made indifferent to enamel as much as possible.

## *1.3 Dental Caries.*

Characteristic for caries is the loss of mineral from the tooth (Groeneveld and Arends 1975). Although the model used in this study resembles mostly the so called smooth surface caries, the mechanism for caries on the molecular level may well be equal for the anatomically different forms of this disease, except for decay of developmental pits which occur sometimes in enamel. Therefore, the following statements will generally hold. For a tooth to be affected by caries a number of conditions must be fulfilled simultaneously (Kõnig, 1971).

1. The tooth must, at least partially, be covered by a layer of acidogenic micro organisms.
2. The microflora must receive nutrients which enable it to maintain and to produce organic acids like lactic acid and acetic acid.
3. The physical and chemical properties of the enamel must be such that it is susceptable to the acids produced.
4. A complex of other host factors must be favorable (among which probably immunological factors).
5. A period over which all the above mentioned factors are favourable for caries simultaneously, must not be too short and such periods must occur at a sufficient frequency.

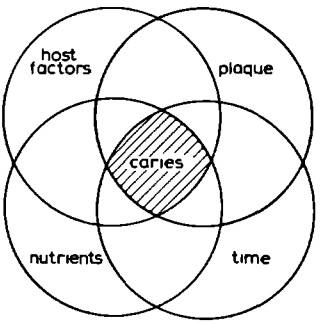


Fig. 1.3.1. Venn diagram illustrating the combination of conditions which is necessary for caries.

Figure 1.3.1 is a Venn diagram illustrating the conditions for caries. The two conditions three and four that are host determined are combined to one in the diagram .

One of the most striking phenomena during caries of the smooth surfaces is that the demineralisation of the enamel does not start at the surface but at a depth of about 40 µm under the surface. So the initial carious lesion has a characteristic, relatively intact surface layer covering a more or less severely demineralised lesion (plate 1.3.1).

The first theory that tried to explain the development of caries was formulated by Miller (1883). He proposed that acidogenic bacteria on the tooth are responsible for its decay. This theory, however, could not explain the occurrence of the subsurface demineralization. Von Bartheld (1961) developed a system in which he could obtain caries like lesions in teeth in vitro. The solutions he used consisted of an acidified gel, which was supposed to be a model for the plaque. The gel was used below its isoelectric point. In the opinion of von Bartheld the establishment of a Donnan equilibrium between gel and enamel, due to the positively charged macromolecules of the gel, was the cause of the demineralization being a subsurface rather than an etch-type one. Later, however, other investigators replaced the charged gel by a neutral one and obtained comparable results (Groeneveld and Arends, 1975).

According to Driessens (1973) a combination of a gradient in the solubility product of the apatite from lower at the surface to higher at greater depth and a remineralizing influence of the saliva during rest periods of the cyclic metabolic activity of the plaque could be responsible for the stability of the surface layer. Langdon (1973), however, obtained caries like lesions in compressed pellets of polycrystalline synthetic hydroxylapatite in which a significant gradient of the solubility product is improbable. On the basis of these observations and

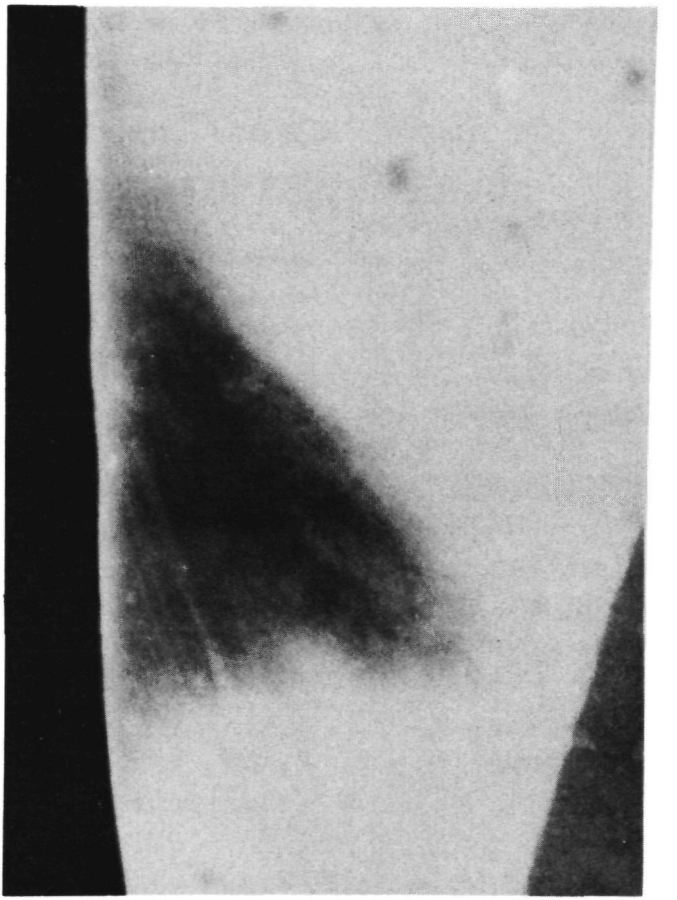

Plate 1.3.1 Microradiograph of a cross section of a natural carious lesion. From left to right: the relatively intact surface layer, the body of the lesion and the not affected rest of the tooth. (By courtesey of Dr. A. Groeneveld).

the results of our computer simulations of caries, as described in chapter 6, we think that the minimum set of conditions necessary to obtain artificially a caries like lesion are

(1) there is a gradient in either the solubility product of enamel or the dissolution rate constant of enamel or the porosity of it,

(2) that, at the pH of the buffer, the buffer capacity of the weak acid is higher than that of the phosphate present in it.

These assumptions are in agreement with the experiments of Moreno (1974) in which he found that if the pH of the buffer which contained no gel was below 4.8 and the calcium and phosphate concentrations are 50 percent or more of those expected at saturation with dental enamel, a subsurface demineralisation occurred. From the data given by Moreno it can be calculated that the buffers used were saturated with respect to DCPD. Moreno concluded from his experiments that the formation of DCPD, which at pH < 4.8 is more stable than apatite, is a necessary condition for the occurrence of a subsurface lesion. Groeneveld and Arends (1975), however, obtained subsurface demineralization using a 6 percent hydroxylethylcellulose gel at pH = 5, a pH at which apatite is probably more stable than DCPD. In this study (chapter 7) we also obtained carieslike lesions using acetate buffers at pH = 5. These buffers were 60 percent or more saturated with synthetic hydroxylapatite. Measurements by van der Hoeven (1977) show that at the interface of an active plaque and enamel the inorganic phosphate concentrations are considerably higher than in saliva, indicating that the liquid phase at this interface is, despite its lower pH, close to saturation.

## *1.4 Decreasing the Caries Sensitivity of Tooth Enamel.*

In the previous section the conditions were mentioned which must be simultaneously favourable for the development of caries. Preventing one of these factors of becoming favourable can in principle reduce caries. In the next chapters we shall try to get an impression of how the physicochemical properties of enamel can be changed in order to reduce the caries sensitivity of enamel.

During an acid attack three types of chemical processes play a role within the enamel.

1. Diffusion of ions and molecules.
2. Dissolution and recrystallization of mineral.
3. Complexation between the various ions in the pore solution.

In the caries process acid must be transported into the enamel and calcium and phosphate must leave the site of the developing lesion. This means that a decrease of the rates of transport will probably decrease the rate of the caries process. In the next chapters we shall restrict our attention to these transport processes. The dissolution process can be retarded by lowering the solubility product of the apatite by for instance fluoride incorporation or by poisoning the apatite surface by for instance a diphosphonate (Wöltgens, 1975).

The rates of the complexation reactions are not susceptible to significant changes in the range of temperatures occurring in the mouth.

In this investigation a method is described to quantify this ionselectivity. The influence of several compounds on the selectivity of enamel is measured and its possible influence on caries is estimated by computer simulations.

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# 2 TRANSPORT PROCESSES IN CHARGED MEMBRANES

## *2.1 Introduction*

Enamel behaves like a charged membrane (section 1.4). The theories which describe the transport processes through charged membranes can be divided into three groups (Laksminarayanaiah, 1969) on the basis of the type of flux equations they use. The first group uses the Nernst-Planck flux equation. In this theory the driving force is assumed to be the gradient of the electrochemical potential. This electrochemical potential is the sum of the chemical and the electrical potential:

$$\mu_{e,i} = \mu_i + Z_i F E \qquad 2.1.1$$

Its gradient is:

$$X_i = \frac{d\mu_i}{dx} + Z_i F \frac{dE}{dx} \qquad 2.1.2$$

The rate which a particle gets under the influence of this force is proportional to the force. The flux equals the product of the rate of the particles and their concentration. If we introduce $RT \ln a_i$ for the chemical potential the flux becomes

$$J_i = - C_i \frac{RT}{k_i} \frac{d\ln a_i}{dx} - Z_i F \frac{C_i}{k_i} \frac{dE}{dx} \qquad 2.1.3$$

If $\frac{RT}{k_i}$ is replaced by $D_i$ (the Einstein relation), this formula becomes Fick's first law for infinite dilution ($C_i = a_i$) and neutral particles ($Z_i = 0$)

$$J_i = - D_i \frac{dC_i}{dx} \qquad 2.1.4$$

Or, with $a_i = f_i C_i$, it becomes the Nernst-Planck Flux equation.

$$J_i = - D_i \left\{ \frac{dC_i}{dx} + C_i \frac{d\ln f_i}{dx} + Z_i C_i \frac{F}{RT} \frac{dE}{dx} \right\} \qquad 2.1.5$$

This equation can be extended by a term for the flux due to convection and one for the flux due to a pressure gradient (Schlögl, 1964a).

The second group uses the principles of the thermodynamics of irreversable processes. The flux equation in this theory is:

$$J_i = \sum_k L_{ik} \chi_k \quad (i,k = 1,2 \ldots\ldots n) \qquad 2.1.6$$

in which $L_{ik}$ are the phenomenological coefficients, $\chi_k$ the generalized forces and n the number of components including the solvent. If the interactions of species i with species k are neglected ($L_{ik} = 0$ if $\neq k$) and if there is no gradient in temperature and pressure the flux equation can be put into the form of the Nernst-Planck equation (Schlögl, 1964a).

In the third group of membrane theories the membrane is considered as a series of potential energy barriers. The flux equation that is assumed for each barrier is:

$$J = K_1 C_1 \lambda_1 - K_2 C_2 \lambda_2 \qquad 2.1.7$$

in which $\lambda$'s are the mean jump distances of the barrier. The first term on the right is the forward flux and the second the backward flux. The following expression stands for $K_1$

$$K_1 = \frac{RT}{Nh} e^{-\Delta F/RT} \qquad 2.1.8$$

in which $\Delta F$ is the free energy of activation necessary to pass the barrier. For $K_2$ an analogous expression is assumed. If all parameters $K_j$ are assumed to be equal to $K$ and $\lambda_j = \lambda$ for all j then the flux equation becomes

$$J = K \lambda^2 \frac{dC}{dx} \qquad 2.1.9$$

which is Fick's first law with $D = - K \lambda^2$ (Laksminarayanaiah, 1969b).

As the second and third group of theories become equivalent

to the group of theories based on the Nernst-Planck equation with the assumptions mentioned above we decided to investigate if our calculations could be done with the Nernst-Planck type of equations.

The fact that enamel behaves like a charged membrane (chapter 1.4) means that the walls of the pores contain fixed ionized groups of which either the cationic or anionic groups may be larger in number, giving the membrane a net positive or negative charge. As electroneutrality must be maintained the following relation holds (Schlögl, 1964b):

$$\Sigma z_i \bar{C}_i + \omega \bar{X} = 0 \qquad 2.1.10$$

This expression, together with an analogous relation for the bulk without the fixed charge term, shows that there is another distribution of mobile ions in the pores than in the bulk. Donnan (1924) formulated a relation between the activities of the various ions in the solution and in the membrane. Supposing that, in equilibrium, the electrochemical potential of each ion is the same inside and outside the membrane, he arrived at the following expression for each ionic species (Helfferich, 1962):

$$\bar{a}_i = a_i \, r^{z_i} \qquad 2.1.11$$

This distribution will cause a diffusion potential across the interface. This so called Donnan potential will be (Helfferich, 1962):

$$E_D = - \frac{RT}{z_i F} \ln \frac{\bar{a}_i}{a_i} = - \frac{RT}{F} \ln r \qquad 2.1.12$$

In the next sections the Nernst-Planck flux equation (2.1.4) and the Donnan relation (2.1.10 and 2.1.11) are supposed to be valid. When activity coefficients have to be calculated we shall

use the same formulas inside the membrane as in the bulk. Thereby the fixed charge is supposed to contribute to the ionic strength of the pore solution only, whereas other possible influences as for instance on the dielectric constant of the solution are ignored.

Important errors due to the assumption mentioned are only to be expected in systems in which the fixed charge concentration is high as compared to the solution concentration, if the solution concentrations are high and if the membrane has a high permeability. Neither of these cases is true for enamel,

(1) it is only slightly ionselective;

(2) the concentrations of the solutions to be used are always less than 0.5 mol $l^{-1}$, and

(3) the permeability of the enamel is very low (section 1.1).

A full discussion of these points can be found in a monograph of Schlögl (1964c) and a paper of Meares (1973).

## *2.2 Membrane potential*

In this section a set of formula will be derived for the e.m.f. of a concentrationcell in which a charged membrane separates two compartments with an electrolyte solution.

For each ion in the system a Nernst-Planck equation can be formulated.

$$J_i = - \bar{D}_i \bar{C}_i \{ \frac{d\ln\bar{C}_i}{dx} + \frac{d\ln\bar{f}_i}{dx} + z_i \frac{F}{RT} \frac{d\bar{E}}{dx} \} \qquad 2.2.1$$

If there is no external electrical connection between the two compartments the total charge, transported by the ion fluxes will be zero:

$$\Sigma z_i J_i = 0 \qquad 2.2.2$$

If the system contains only one cationic and one anionic species the two formula 2.2.1 together with the condition 2.2.2 can be rearranged to

$$d\bar{E} = -\frac{RT}{F}\,\frac{z_+\bar{D}_+\bar{C}_+ d(\ln\bar{C}_+) + z_-\bar{D}_-\bar{C}_- d(\ln\bar{C}_-)}{z_+^2\,\bar{D}_+\bar{C}_+ + z_-^2\,\bar{D}_-\bar{C}_-}$$

$$-\frac{RT}{F}\,\frac{z_+\bar{D}_+\bar{C}_+ d(\ln\bar{f}_+) + z_-\bar{D}_-\bar{C}_- d(\ln\bar{f}_-)}{z_+^2\,\bar{D}_+\bar{C}_+ + z_-^2\,\bar{D}_-\bar{C}_-} \qquad 2.2.3$$

If it is assumed that both the fixed charge and the diffusion coefficients are constants, that is, they are independent of the concentrations and of the place in the membrane, this relation can be integrated. Using the electroneutrality condition 2.1.10 the first term of 2.2.3 becomes on integration:

$$\bar{E}_C = -\frac{RT}{F}\,\frac{\bar{D}_+ - \bar{D}_-}{z_+\bar{D}_+ - z_-\bar{D}_-}\,\ln\left\{\frac{z_+^2\,\bar{D}_+\bar{C}_+'' + z_-^2\,\bar{D}_-\bar{C}_-''}{z_+^2\,\bar{D}_+\bar{C}_+' + z_-^2\,\bar{D}_-\bar{C}_-'}\right\} \qquad 2.2.4$$

We shall call this term the concentration term of the diffusion potential. The second term of 2.2.3 becomes on integration

$$\bar{E}_A = -\frac{RT}{F}\int_{'}^{''}\frac{z_+D_+\bar{C}_+ d(\ln\bar{f}_+) + z_-D_-\bar{C}_- d(\ln\bar{f}_-)}{z_+^2\,\bar{D}_+\bar{C}_+ + z_-^2\,\bar{D}_-\bar{C}_-} \qquad 2.2.5$$

If the gradients of the activity coefficients become zero, which means that the activity coefficients are constant throughout the entire membrane then the term 2.2.5 vanishes. We shall call this term $\bar{E}_A$ the activity correction term of the diffusion potential. This activity correction term cannot be solved unless a relation between the concentration and the activity coefficients is available. For diluted solutions ($C < 1$ mol $l^{-1}$) the

formula of Guggenheim (Robinson and Stokes, 1970) gives a good approximation:

$$\log \bar{f}_{\pm} = - A \, |z_+ z_-| \, \frac{\sqrt{\bar{I}}}{1 + \sqrt{\bar{I}}} + b \, \bar{I} \qquad 2.2.6$$

In which the ionic strength is assumed to be

$$\bar{I} = \tfrac{1}{2} \{ \bar{X} + \Sigma z_i^2 \, \bar{C}_i \} \qquad 2.2.7$$

If we assume that (which is not entirely true for the formula 2.2.6)

$$\ln \bar{f}_+ = - \frac{z_+}{z_-} \ln \bar{f}_{\pm}$$

$$\text{and } \ln \bar{f}_- = - \frac{z_-}{z_+} \ln \bar{f}_{\pm} \qquad 2.2.8$$

then formula 2.2.5 can be rearranged to

$$\bar{E}_A = \frac{RT}{F} \int_{'}^{''} \frac{\frac{z_+^2}{z_-} \bar{D}_+ \bar{C}_+ + \frac{z_-^2}{z_+} \bar{D}_- \bar{C}_-}{z_+^2 \bar{D}_+ \bar{C}_+ + z_-^2 \bar{D}_- \bar{C}_-} \, d \ln \bar{f}_{\pm} \qquad 2.2.9$$

This integral can be solved by numerical integration, for instance Gauss Quadrature (Appendix A.2, Stoer, 1972a), using a relation for $\bar{f}_{\pm}$ like the Guggenheim formula and the elctroneutrality condition 2.1.10. Of course the integral 2.2.5 can be calculated directly by numerical integration when a more sophisticated formula for the single-ion activity coefficients is desired.

Up to now all concentrations were inner membrane concentrations. In practice however these concentrations are not

known and we have to use the Donnan equilibrium relations 2.1.11 to calculate these inner membrane concentrations from the bulk concentrations. When we use activity coefficients the Donnan relations for the cation and anion become:

$$\bar{C}_+ = C_+ \frac{f_+}{\bar{f}_+} r^{Z_+}$$

$$\bar{C}_- = C_- \frac{f_-}{\bar{f}_-} r^{Z_-} \qquad 2.2.10$$

If we introduce in 2.2.4 the following relations and abbreviations:

$$C_+ = \nu_+ C$$
$$C_- = \nu_- C$$
$$\nu_+ Z_+ = - \nu_- Z_-$$
$$\rho_+ = \frac{f_+}{\bar{f}_+}$$
$$\rho_- = \frac{f_-}{\bar{f}_-} \qquad 2.2.11$$

then the formula can be rearranged to:

$$E_C = - \frac{RT}{F} \frac{\bar{D}_+ - \bar{D}_-}{Z_+\bar{D}_+ - Z_-\bar{D}_-} \ln \left\{ \frac{C'' \left(Z_+\bar{D}_+ \rho_+'' (r'')^{Z_+} - Z_-\bar{D}_-\rho_-'' (r'')^{Z_-}\right)}{C' \left(Z_+\bar{D}_+\rho_+' (r')^{Z+} - Z_-\bar{D}_-\rho_-' (r')^{Z-}\right)} \right\}$$

2.2.12

If we have expressions for the ρ's and r's then the diffusion potential can be calculated using 2.2.9 and 2.2.12. Using the Donnan relations 2.2.10 the electroneutrality condition 2.1.10 and the set of relations and abbreviations 2.2.11 the Donnan distribution coefficients can be calculated from:

$$\rho_+ r^{(Z_+ - Z_-)} + \frac{\omega \bar{X}}{Z_+ \nu_+ C} r^{-Z_-} - \rho_- = 0 \qquad 2.2.13$$

This is a polynomial of degree two or higher in r from which it can be proved that it has always one and never more than one positive real root. The equation 2.2.13 has to be solved numerically. With a good starting value this is efficiently done using Newton-Raphson iteration (Appendix A.1, Stoer 1972b).

As pointed out in section 2.1 the Donnan distribution will give rise to a liquid junction potential across the membrane-bulk interface. The total membrane potential $E_M$ will be the sum of the diffusion potential $\bar{E}$, which is the sum of $\bar{E}_C$ and $\bar{E}_A$ and the Donnan potentials caused by the Donnan equilibria at both interfaces $E_D'$ and $-E_D''$

$$E_M = \bar{E}_C + \bar{E}_A + E_D' - E_D'' \qquad 2.2.14$$

introducing $E_D = E_D' - E_D''$ we get for the total Donnan potential with 2.1.12

$$E_D = \frac{RT}{F} \ln \frac{r''}{r'} \qquad 2.2.15$$

If the solution is not a uni-univalent electrolyte and if it is not assumed that the activity coefficients are constant throughout the system, numerical procedures have to be used to calculate the membrane potential. In appendix B the computer program TMS4 is described, which calculates the membrane potential as a function of input variables.

The formula for the activity coefficients that is used is 2.2.6, the Guggenheim formula. The adjustable parameter b is calculated using a least squares procedure and tabulated values for the activity coefficients.

For uni-univalent electrolytes the set of formulas becomes considerably more simple. If we assume that the ratio's of the activity coefficients of the ions in the bulk and the membrane are the same for the cations as for the anions, $\rho_+ = \rho_-$, then the concentration term 2.2.12 becomes:

$$\bar{E}_C = -\frac{RT}{F}\,\frac{\bar{D}_+ - \bar{D}_-}{\bar{D}_+ + \bar{D}_-}\,\ln\left\{\frac{C''}{C'}\,\frac{(\bar{D}_+ r'' + \bar{D}_-/r'')}{(\bar{D}_+ r' + \bar{D}_-/r')}\right\} - \frac{RT}{F}\,\frac{\bar{D}_+ - \bar{D}_-}{\bar{D}_+ + \bar{D}_-}\ln\left(\frac{\rho''}{\rho'}\right) \qquad 2.2.16$$

The activity correction term 2.2.9 becomes:

$$\bar{E}_A = -\frac{RT}{F}\int_{'}^{''}\frac{\bar{D}_+\bar{C}_+ - \bar{D}_-\bar{C}_-}{\bar{D}_+\bar{C}_+ + \bar{D}_-\bar{C}_-}\,d\ln\bar{f}_{\pm} \qquad 2.2.17$$

The equation for the Donnan distribution coefficients 2.2.13 becomes:

$$r^2 + \frac{\omega\bar{X}}{\rho C}\,r - 1 = 0 \qquad 2.2.18$$

for both r' and r".

This is not a simple second degree equation, as might be expected at first sight, because the ρ contains both bulk and inside concentrations and thus also the r itself.

As can be seen clearly from these three formula the activity coefficients appear as ratio's or gradients only. If these ratio's are all set equal to one and the gradients to zero then the second term of 2.2.16 and the activity correction term vanish and the Donnan equation becomes a simple second degree

equation which can be solved directly.

$$r = \sqrt{1 + \left(\frac{\omega\bar{X}}{2\,C}\right)^2} - \frac{\omega\bar{X}}{2\,C} \qquad 2.2.19$$

Under this assumption, which is less severe than neglecting activity coefficients entirely, the formula 2.2.15, 2.2.16 and 2.2.17 become, on rearrangement, equivalent with the first theoretical treatment of the potential across charged membranes by Teorell (1953) and Meyer and Sievers (1936).

The various terms of the formula of this chapter will be illustrated for a uni-univalent electrolyte.

Example:

solution : $C' = 0.1$ mol $l^{-1}$, $C'' = 0.02$ mol $l^{-1}$

membrane: $\omega\bar{X} = -0.01$ eq $l^{-1}$, $\bar{D}_+/\bar{D}_- = 1.5$

Guggenheim formula: $A = 0.5115$, $b = 0.055$

$T = 298.16$ K

$R = 8.314$ J $K^{-1}$ $mol^{-1}$

$F = 96.494\ 10^3$ C $mol^{-1}$

$(RT/F = 25.69$ mV)

then

$E_M$ = 12.42 (13.00) mV

$\bar{E}_C$ = 7.89 ( 7.93) mV

$\bar{E}_A$ = -0.45 ( 0.0 ) mV

$E_D$ = 4.99 ( 5.07) mV

$\rho'_+$ = 1.005 ( 1.00) $r'$ = 1.051 (1.051)

$\rho''_+$ = 1.015 ( 1.00) $r''$ = 1.276 (1.281)

The terms in parentheses are the ones obtained if the activity coefficients are assumed to be constant throughout the entire system.

## *2.3 General Integration of the Nernst Planck Flux Equation.*

In the previous section a method is given to calculate the steady state membrane potential of a charged membrane, if the solution is a simple electrolyte. In a number of cases it is necessary to know this membrane potential and the fluxes of the various components in a more complex system. It can also be of importance to know the concentrations on points within the membrane. This makes it necessary to find a general method to integrate the Nernst-Planck flux equation 2.1.5.

Schlögl (1954) has given a method for integrating the Nernst-planck equation for a general electrolyte system. His treatment is analytical but contains a parameter function that has to be calculated numerically in most cases. Disadvantages of Schlögl's method are that the parameter function has several points in which it is not defined. Thus numerical solution of this nonlinear function can be difficult. Furthermore, it is not possible to introduce activity coefficients in Schlögl's method.

We have chosen for an entirely numerical solution of this problem. The basic point in this new method is that the concentrations of the compounds and the potential as a function of the place in the membrane are represented by polynomials of a higher degree. If the coefficients of these polynomials are known the gradients of the concentration and of the potential can easily be calculated as well as, when desired, the gradient of the logarithm of the activity coefficients. Thus the fluxes can be calculated using the Nernst-Planck equation 2.1.5 if we

find some way to calculate the coefficients of the polynomials. For the solution of the problem we define a number of points within the membrane (including the two boundaries) at which a number of conditions must be fullfilled.

These conditions are:

(1) for every ionic species i at each point j:

$$0 = J_{ij} - [\ -\bar{D}_{ij}\,\bar{C}_{ij}\,\{\ \frac{d\ \ln\ \bar{C}_{ij}}{dx} + \frac{d\ \ln\ \bar{f}_{ij}}{dx} + z_i\,\frac{F}{RT}\,\frac{d\ \bar{E}_j}{dx}\ \}\ ] \qquad 2.3.1$$

(2) at each point j:

$$0 = \omega\bar{X}_j + \sum_i z_i\,\bar{C}_{ij} \qquad 2.3.2$$

(3) at each point j:

$$0 = J_E - \sum_i z_i\,J_{ij} \qquad 2.3.3$$

(4) for each component and at each point the condition of a steady state:

$$0 = \frac{d\bar{C}_{ij}}{dt} = \frac{dJ_{ij}}{dx} \qquad 2.3.4$$

This last relation means that the fluxes of each component are constant and thus the relations 2.3.3 are reduced to one relation.

If we have n components and m points the number of conditions is mn + m + 1. Each condition is a relation between the concentrations and the potential and thus of the coefficients of the polynomials which describe the concentrations and the potential as a function of x. Thus in fact we have created mn + m + 1 functions with the unknown coefficients of n + 1 polynomials and n unknown fluxes. If we chose the degrees of the polynomials such that the total number of coefficients and

fluxes is less than or equal to mn + m + 1,we have a system of mn + m + 1 nonlinear functions with mn + m + 1 or less unknown parameters. If we minimize the sum of squares of the functions 2.3.1, 2.3.2 and 2.3.3 we have a solution for our problem. If the degrees of the polynomials are such that the number of unknown parameters equals the number of functions this minimum will be zero and the conditions are fullfilled exactly in the m points. Using this principle of solving the problem of a general integral of the Nernst-Planck equations we wrote a computer program. In this program the concentrations and the potential are not described by one polynomial but by a lineair combination of legendre polynomials (Stoer, 1972c), which has several numerical advantages. The nonlinear least squares problem into which our problem is converted, is solved using tne algorithm of Powell (1965). For more details of the program, which is called FLUX see appendix C.

As an example of a solution that can be obtained with the program FLUX we take the same system as Schlögl (1954) did. The membrane in the concentration cell has a positive charge, the left solution is a $MgSO_4$ solution and the right one contains $K_2SO_4$ and $Na_2SO_4$. The thickness of the membrane is 0.1 cm. To make a comparison possible between the results of FLUX and those of Schlögl we assume the fixed charge and the diffusion coefficients constant and the activity coefficients to be unity.

Example

$\omega\bar{X} = 1.0 \quad mol.l^{-1}$

$\bar{D}_{Mg^{++}} = 3.010^{-6} \quad cm^2 \, s^{-1}$

$\bar{D}_{SO_4^{=}} = 3.010^{-6} \quad cm^2 \, s^{-1}$

$\bar{D}_{K^+} = 5.010^{-6} \quad cm^2 \, s^{-1}$

$\bar{D}_{Na^+} = 3.010^{-6} \quad cm^2 \, s^{-1}$

$\delta$ = 0.1 cm (thinkness of membrane)

$J_E$ = 65 mA $cm^2$ (external current density)

R = 8.314 J $K^{-1}$ $mol^{-1}$

T = 298.16 K

F = 96.494 $10^3$ C $mol^{-1}$

The bulk concentrations are mentioned in table 2.3.1 together with the inner membrane concentrations and the potential as a function of the depth.

Table 2.3.1.

Concentrations and potential as function of the distance in the membrane as calculated with the program FLUX from the example of Schlögl (1954)

| distance | concentrations mol $l^{-1}$ | | | | potential |
|---|---|---|---|---|---|
| in membrane | $Mg^{++}$ | $SO_4^{=}$ | $K^+$ | $Na^+$ | mV |
| bulk | 0.707 | 0.707 | 0.000 | 0.000 | 0 |
| 0.00 | 0.500 | 1.000 | 0.000 | 0.000 | 4.45 |
| 0.01 | 0.226 | 0.870 | 0.215 | 0.073 | 14.58 |
| 0.02 | 0.086 | 0.814 | 0.340 | 0.115 | 26.94 |
| 0.03 | 0.030 | 0.800 | 0.404 | 0.137 | 40.29 |
| 0.04 | 0.010 | 0.803 | 0.438 | 0.148 | 54.31 |
| 0.05 | 0.003 | 0.810 | 0.459 | 0.155 | 68.13 |
| 0.06 | 0.001 | 0.817 | 0.472 | 0.160 | 81.83 |
| 0.07 | 0.000 | 0.823 | 0.482 | 0.163 | 95.40 |
| 0.08 | 0.000 | 0.827 | 0.490 | 0.166 | 108.87 |
| 0.09 | 0.000 | 0.831 | 0.495 | 0.167 | 122.25 |
| 0.10 | 0.000 | 0.843 | 0.500 | 0.169 | 135.59 |
| bulk | 0.000 | 0.453 | 0.677 | 0.229 | 127.75 |

The fluxes are:

$$J_{Mg^{++}} = 1.39 \cdot 10^{-10} \text{ mol cm}^{-2} \text{ s}^{-1}$$

$$J_{SO_4^{=}} = 2.58 \cdot 10^{-7} \text{ mol cm}^{-2} \text{ s}^{-1}$$

$$J_{K^{+}} = -1.31 \cdot 10^{-7} \text{ mol cm}^{-2} \text{ s}^{-1}$$

$$J_{Na^{+}} = -2.66 \cdot 10^{-8} \text{ mol cm}^{-2} \text{ s}^{-1}$$

Characteristic for this example is the dip in the sulphate concentration and the low magnesium flux. The dip disappears when the external current becomes, in absolute value, lower. Then the magnesium flux becomes normal too.

The method of finding a general integral of the Nernst-Planck flux equations yields results that are in quantitative agreement with those of the method of Schlögl. It has a number of advantages over Schlögl's method:

(1) activity coefficients can be introduced, which is done in the computer program,

(2) the diffusion coefficients and

(3) the fixed charge can be a function of the concentrations and the place in the membrane

(4) pressure and convection terms can be introduced without making the program much more complicated.

## 2.4 *Calculation of Membrane Properties from Membrane Potentials.*

In the previous sections of this chapter a description is given of how the various membrane phenomena can be calculated from the properties of the membrane and the surrounding electrolytes. In practice, however, it is more common that the phenomena can be measured than the properties. Thus we will have

to calculate the properties from the measurements of, for instance, fluxes and potentials. We shall restrict ourselves to how and which membrane properties can be calculated from electromotive force(emf) measurements with single electrolytes.

A closer look at the set of formulas for the membrane potential 2.2.4, 2.2.9 and the Donnan equilibrium 2.2.13 reveals that the membrane potential is determined by four mathematically independent variables: the two bulk concentrations,the ratio of the two diffusion coefficients ($\bar{D}_+/\bar{D}_-$) and the fixed charge. In an experiment in which the membrane potential is measured the bulk concentrations will be known. So the ratio of the diffusion coefficients and the fixed charge remain as independent and unknown parameters. This means that the emf must be measured for at least two different pairs of bulk concentrations to get a system of two or more non-linear equations from which it should be possible to calculate the two unknown membrane properties.

We have developed a procedure with which we can calculate the ratio of the diffusion coefficients of the cation and anion inside the membrane and the fixed charge of the membrane. We shall illustrate this procedure for the most simple case of a uni-univalent electrolyte, ignoring activity coefficients. We shall call the emf that can be calculated with the formula 2.2.16 and 2.2.19 the expected membrane potential $\hat{E}$

$$\hat{E} = -\frac{RT}{F}\left\{\frac{\frac{\bar{D}_+}{\bar{D}_-}-1}{\frac{\bar{D}_+}{\bar{D}_-}+1}\right\}\ln\left\{\frac{C''\left(\frac{\bar{D}_+}{\bar{D}_-}r''+\frac{1}{r''}\right)}{C'\left(\frac{\bar{D}_+}{\bar{D}_-}r'+\frac{1}{r'}\right)}\right\}-\frac{RT}{F}\ln\left(\frac{r''}{r'}\right) \qquad 2.4.1$$

$$r = \sqrt{1 + \left(\frac{\omega\bar{X}}{2C}\right)^2} - \frac{\omega\bar{X}}{2C} \qquad 2.4.2$$

for both r' and r"

If we have done emf measurements with n different pairs of solutions with concentrations $C_i'$ and $C_i''$ then we have n emf's $E_i$. Our object is to calculate those values for $\omega\bar{X}$ and $\bar{D}_+/\bar{D}_-$ for which the sum of squares of the differences between expected and measured emf's is minimal or:

$$\left(\frac{\partial \sum_{i=1}^{n} (\hat{E}_i - E_i)^2}{\partial\, \omega\bar{X}}\right)_{\frac{\bar{D}_+}{\bar{D}_-}} = 0$$

and

$$\left(\frac{\partial \sum_{i=1}^{n} (\hat{E}_i - E_i)^2}{\partial\, (\frac{\bar{D}_+}{\bar{D}_-})}\right)_{\omega\bar{X}} = 0 \qquad 2.4.3$$

The problem is now reduced to solving a set of two equations with two unknown parameters. The formula for the membrane potential are, however, too complicated to calculate the two derivatives directly, especially if activities are not neglected. For finding the desired minimum of the sum of squares we have chosen the method of Powell (1965) for minimizing the sum of squares of a system of non-linear functions in several unknown parameters without calculating derivatives. This is an iterative procedure which, apart from a number of controll varia-

bles, needs starting values for the unknown parameters and a procedure for calculating the membrane potentials. For each step of an iteration the procedure of Powell calculates better values for $\omega\bar{X}$ and $\bar{D}_+/\bar{D}_-$ until some convergence criterion is satisfied. The computer program, TMS3, which is based on this principle is described in more detail in appendix D.

The minimum of the sum of squares, say Q, is a measure for the fit of the mathematical model to the experimental values which can be expressed in terms of a standard error in the measurements

$$s_E = \sqrt{\frac{Q}{n-2}} \qquad 2.4.4$$

where n-2 is the number of degrees of freedom. With this standard error and the variance covariance matrix, which is calculated in the procedure of Powell, the standard errors in $\bar{D}_+/\bar{D}_-$ and $\omega\bar{X}$ can be calculated. If $\Gamma$ is the 2xn matrix with elements.

$$\gamma_{i1} = \left(\frac{\partial \hat{E}_i}{\partial \omega\bar{X}}\right)_{\bar{D}_+/\bar{D}_-} \quad \text{and} \qquad 2.4.5$$

$$\gamma_{i2} = \left(\frac{\partial \hat{E}_i}{\partial \bar{D}_+/\bar{D}_-}\right)_{\omega\bar{X}} \qquad i = 1, 2 \ldots n \qquad 2.4.6$$

then the variance covariance matrix is the two by two matrix

$$H = \frac{Q}{n-2}\,(\Gamma^T\Gamma)^{-1} \qquad 2.4.7$$

and

$$s_{\omega\bar{X}} = \sqrt{h_{1,1}} \qquad 2.4.8$$

$$s_{\bar{D}_+/\bar{D}_-} = \sqrt{h_{2,2}} \qquad 2.4.9$$

where $h_{1,1}$ and $h_{2,2}$ are the two diagonal elements of H.

Up to now we have assumed that the solution contains only two ionic species. In our experiments, however, more species are present. Due to the special properties of the membranes, which consist for almost 90 percent of apatite (chapter 1), it is highly desirable to use electrolyte solutions that are buffered and saturated with respect to enamel (section 3.6). As all solutions used had a pH of 7.4 the calcium and phosphate concentrations will be less than 1 mmol $l^{-1}$ but the buffer component which is for 50 percent dissociated at pH = 7.4 had a concentration of 2 mmol $l^{-1}$ which is 20 percent of the concentration of the most diluted solution used in the experiments. Calculations with the program FLUX (section 2.3) have shown that the influence of the buffer on the diffusion potential is negligable, the contribution to the Donnan potential is larger but still small. Correcting the Donnan potential for the buffer concentration is, however, very simple and incorporated in the computer programs (TMS3 and TMS4 see appendices B and D). The emf of the example of section 2.2 is without corrections for the buffer 12.34 mV, with correction on the Donnan potential only 11.97 mV and with correction on both the Donnan potential and the diffusion potential 11.94 mV. These three values have been calculated with the program FLUX (section 2.3, appendix C) with correction for activity coefficients. The first value is the same as that of the example of section 2.2 with activity coefficients. It shows the good agreement between the two entirely different ways of calculating the membrane potential. In fact the difference between the two values is caused by the assumptions 2.2.8 which introduce a small error in the emf of about 0.6 percent if the Guggenheim formula for activity coefficients (2.2.6) is used. The assumptions 2.2.8 are not used in the general integration of the Nernst-Planck equations.

## 2.5 *Interpretation of Measurements from the Literature.*

As early as in 1926 Amberson, Williams and Klein (1926) carried out emf measurements on dental enamel. Waters did a great number of emf measurements on enamel caps of whole human teeth, sections of human enamel and sections of synthetic hydroxylapatite (Waters, 1968, 1971, 1975). These emf measurements have were as input for our computer program (Van Dijk et al. 1977). The results of a typical experiment of Waters (1971) are tabulated in tables 2.5.1 and 2.5.2 and plotted in figure 2.5.1. The fifth and sixth column of the table 2.5.1 contain the calculated emf's that give the best fit to

Table 2.5.1.

The emf's of a concentration cell with human enamel measured with two different electrolyte at various concentrations (Waters, 1971) and calculated with the program TMS3.

| concentrations mol $l^{-1}$ | | emf in mV measured | | calculated | |
|---|---|---|---|---|---|
| C' | C" | KCl | NaCl | KCl | NaCl |
| 0.1 | 0.1 | - 0.3 | 1.6 | 0.0 | 0.0 |
| 0.1 | 0.05 | 2.7 | 1.1 | 3.6 | 0.7 |
| 0.1 | 0.03 | 6.6 | 1.4 | 7.2 | 2.1 |
| 0.1 | 0.02 | 10.7 | 3.3 | 10.9 | 3.9 |
| 0.1 | 0.01 | 21.9 | 10.4 | 19.5 | 9.2 |
| 0.1 | 0.005 | 30.8 | 17.7 | 30.7 | 17.5 |
| 0.1 | 0.002 | 45.1 | 30.2 | 46.4 | 30.7 |
| estimated st-error | | 1.2 | 1.0 | 1.3 | 1.0 |

Table 2.5.2.

Results of calculations on the data of table 2.5.1; the figures in parentheses are the calculated standard errors in the fixed charge ($\omega\bar{X}$) and the ratio of the diffusion coefficients ($\bar{D}_+/\bar{D}_-$).

| | KCl | NaCl |
|---|---|---|
| $\omega\bar{X}$ meq $l^{-1}$ | 16 (4) | 12 (2) |
| $D_+/D_-$ | 1.2 (0.2) | 0.92 (0.07) |

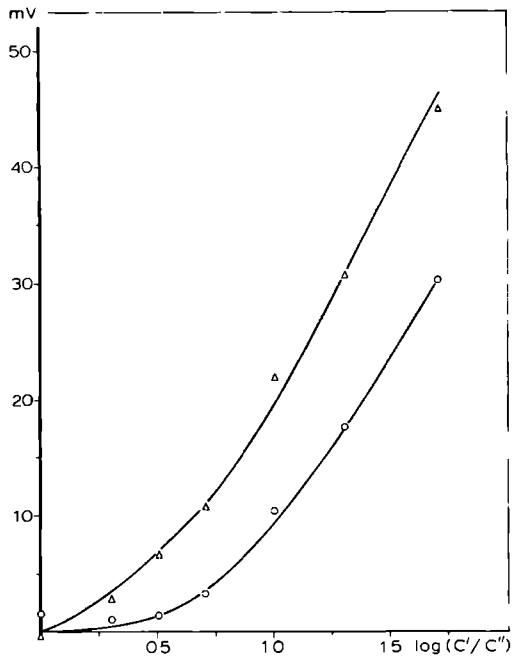


Figure 2.5.1.
The emf's of Table 2.5.1. plotted as a function of log (C'/C"). Triangles are the KCl measurements and circles the NaCl measurements. The drawn lines follow the calculated emf's. Potentials are in millivolts.

the measured emf's. The standard errors in the columns three and four are estimated from the data of Waters, the standard errors in the columns five and six are calculated using formula 2.4.4. The figures in parentheses in table 2.5.1 represent the standard errors in the fixed charge and the ratio of $\bar{D}_+$ and $\bar{D}_-$, calculated with the formulas 2.4.8 and 2.4.9 respectively. It follows from this example and all other evaluated measurements of Waters (Van Dijk et al. 1977) that the ratio of the diffusion coefficients can be calculated with a good accuracy from the emf measurements but that the value of the fixed charge is more uncertain. This is due to the fact that, with the relatively low fixed charge concentrations of enamel, the membrane potential is mainly determined by the ratio of the diffusion coefficients.

The calculations on the emf's of table 2.5.1 can of course also be done without making correction for the presence of the buffer and without corrections for the activity coefficients. In table 2.5.3 the calculated standard errors in the emf in mV (formula 2.4.4) are shown in relation to the corrections made for the KCl experiment of Waters. This example is in fact the

Table 2.5.3.
*The effect of considering the presence of the buffer components* and the activity coefficients on the calculated standard error in the emf in mV from the KCl data from table 2.5.1.

| | | buffer considered | |
|---|---|---|---|
| | | yes | no |
| activity coeff. | yes | 1.3 | 2.1 |
| considered | no | 1.4 | 2.2 |

inverse of the example of sections 2.2 and 2.4 and clearly shows the effect of the corrections. The large effect of the buffer is mainly due to the measurement with the two solutions 0.1/0.002 mol $l^{-1}$.

On the basis of the comparison of the standard error in the measurements and the standard error which is calculated from the difference between measured and calculated emf's it is not to be expected that modifications of the model will result in a better fit of the model to the measured data. The results of the experiments which are described in the chapters four and five also support the usableness of the formulas derived in this chapter.

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# 3 EXPERIMENTAL EQUIPMENT AND MATERIALS.

## *3.1 Introduction.*

The experimental set up for the measurement of the selectivity of a slice of enamel consists of four units:

1. The concentration cell for the slice of enamel placed in a thermostat.
2. A pair of reference electrodes, one in either compartment of the cell.
3. An amplifier and a recorder to record the emf of the cell.
4. The electrolyte solutions in both compartments of the cell.

The qualifications to be met for all four units are that they should influence the electrochemical characteristics of the slice of enamel as little as feasable and that the emf recorded is a simple and known function of the potential of the cell. On points in the experimental procedures where it is not possible to avoid influencing the properties of enamel the procedures have been standardized as much as possible in order to make the experimental bias the same for all experiments. The most important of these points were the preparation of the slices of enamel and the application of the electrolyte solutions.

## *3.2 The Concentration Cell.*

The concentration cell (see plate 3.2.1) is made of a commercial PMMA. The volume of either compartment is about 18 ml. The slice of enamel is fixed between two small annular rings. The space around the slice of enamel outside these rings is filled with a tough grease (Apison M) in order to prevent

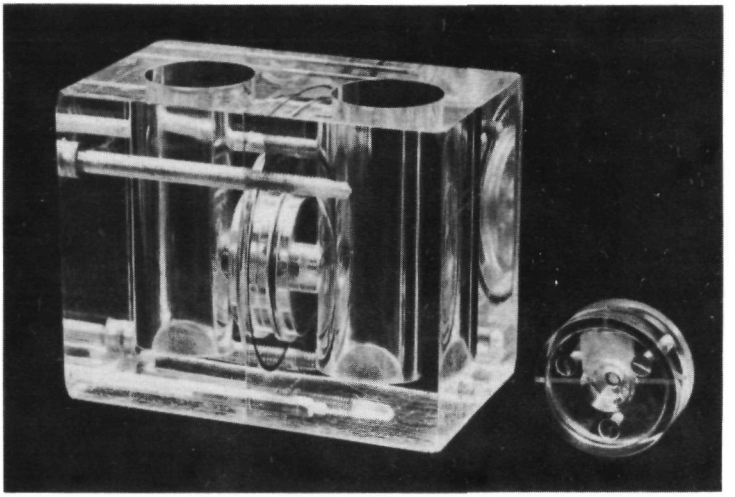

Plate 3.2.1.
Photograph of the concentration cell (left) and the holder for the piece of enamel (right). The holder fits in holes in either compartment and is fixed between rubber rings.

electrolyte leakage from one compartment to the other, which would short circuit the fluxes of ions through the membrane. The cells were tested on leakage from one compartment to the other by the use of radioactive compounds and a metal sheet in place of the enamel. No activity passed the cell (Borggreven, Van Dijk and Driessens, 1977). The construction is a further development of that used by Waters (1970).

## *3.3 Reference Electrodes.*

In order to be able to measure the diffusion potential

across the enamel membrane in the concentration cell, a reference electrode has been placed in either compartment. We have tried out two types of electrodes, the calomel electrode and the silver-silverchloride electrode. The calomel electrode must be connected to the cell by a salt bridge. The advantage of this arrangement is that the emf between the electrodes is equal to that between the two solutions in the cell and thus to the potential over the membrane. A disadvantage is that the porous plug can give rise to erroneous measurements by aging or clogging. Another disadvantage of the commercially available calomel electrodes is that the electrode potential is not the same for all electrodes and can vary up to a few millivolts.

The silver-silverchloride electrode can be placed directly in the solution of the cell as long as these solutions contain chloride ions and no ions that disturb the electrode as bromide does. A disadvantage of this electrode is, however, that the potential between electrode and solution is not independent of the solution composition, but a simple function of the chloride activity of the solution:

$$E_E = \frac{RT}{F} \ln a_{Cl^-} + E_O \qquad 3.3.1$$

The emf between the electrodes does not equal the membrane potential but is the membrane potential plus the difference between the two electrodes potentials.

There are several types of silver-silverchloride electrodes, they can differ in both shape and method of preparation (Ives and Janz, 1961). The ones we have used are of the thermoelectrolytic type. The basis of the electrodes is a small helix of platinum wire. On this wire a paste of silver oxide is applied,

which is decomposed into silver by heating at 450°C. The sphere of porous silver that has been deposited is then subjected to a partial electrolysis in dilluted hydrochloric acid. The layer of silverchloride which is formed contains about 15 percent of the silver which was deposited.

After some experimentations with both types of reference electrodes we have chosen for the Ag/AgCl electrodes for our experiments. The emf measurements with these electrodes had a better reproducability than with the calomel electrodes.

The electrodes were prepared in batches of ten. Electrodes with an electrode potential that differed more than 0.2 mV from one of the other electrodes from the batch, were rejected. At regular time intervals this check was repeated for each pair of one cell.

### *3.4 The Electronic Equipment.*

The emf's to be expected in the experiments range from -100 to +100 mV at the most. The membrane potentials do in general not exceed plus or minus 20 mV depending on the solutions used. These potentials as such are large enough to be registrated directly by a commercial recorder. The ion fluxes, however, that cause the diffusion potential to develop are in the order of magnitude of $10^{-12}$ mol $s^{-1}$, which corresponds, for univalent electrolytes, with a current of a few hundred nanoamperes. Therefore, the input current of the registrating equipment should be very low in order not to disturb the ion fluxes and thus the membrane potential. Each pair of electrodes was connected to a Multipoint-recorder (Honeywell Versaprint) via an electrometer amplifier. The amplifier was used as a voltage

follower, thus acting as an impedance transformer with an extremely high input impedance (< $10^{-14}$ A) and a low output impedance. The active element of the amplifier is a varactor bridge operational amplifier (Analog Devices 311J) of which the inverting input is used to substract an adjustable voltage from the input voltage. A diagram of the amplifier is given in fig. 3.4.1. By this device we were able to limit the output voltage to 10 mV, the full scale of the recorder, independent of the input voltage. The voltage readings of the recorder sheet could be made with an accuracy of 0.1 mV over the whole range from -100 to +100 mV.

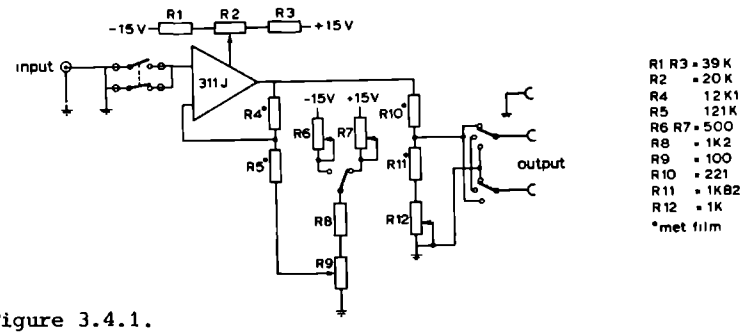


Figure 3.4.1.
Diagram of the electrometer amplifier.

## *3.5 Enamel Sections.*

The slices of dental enamel were prepared from bovine incisors. The permanent teeth of 1.5 to 2 years old cattle were extracted just before eruption. They were cleaned by brushing and rinsing with distilled water. Slices of 200 µm thickness were prepared by sawing parallel to the labial surface. The slices were stored in a 0.002 mol $l^{-1}$ HEPES (2[ 4(2-hydroxy-ethylpiperazinyl-(c)] ethanesulfonic acid) buffer

of pH 7.4 containing 40 μmol $l^{-1}$ chlorhexidine-HCl to prevent bacterial growth.

Before a slice was mounted in a cell it was checked on micro cracks and contamination with dentine both microscopically and radiographically. For the microradiographs a tungsten source was used at 20 kV and 18 mA. After being mounted in the cell they were again checked on micro cracks.

### *3.6 Electrolyte Solutions for emf Measurements.*

The basis of all electrolyte solutions for emf measurements is a 0.002 mol $l^{-1}$ HEPES buffer of pH = 7.4 containing 40 μmol $l^{-1}$ chlorhexidine. This buffer has been in contact with powdered enamel from the same source as the slices of enamel during at least 14 days to get a solution which is more or less saturated with respect to enamel apatite. The electrolyte used for most of the measurements was RbCl. We used RbCl instead of the more common KCl to be able to compare the electrochemical measurements with the radiochemical measurements of Borggreven et al. (1977). The RbCl used was Merck suprapur, all other chemicals were pro analisi. The water used was demineralized and distilled twice.

### *3.7 Agitating the Electrolyte Solution.*

It is common practice in experiments in which membrane potentials are measured to agitate the solution near the membrane (Scatchard and Helfferich, 1956). This will reduce the diffusion film near the membrane surface. In the presence of a film the potential between the two bulk solutions will in general be less than that between the solutions directly adjacent to the membrane. Helfferich (1962) has derived a rule of

thumb which indicates whether or not the influence of the film will be significant. The diffusion will be controlled by the membrane only if:

$$\frac{D\ C\ d}{\bar{D}\ \bar{C}\ \delta} >> 2 \qquad 3.7.1$$

The charge of the enamel membranes is such that the ratio $C/\bar{C}$ will, in our experiments, not differ much from one.
From tracer measurements it is known (Borggreven, Van Dijk and Driessens, 1977) that the ratio $D/\bar{D}$ is about $10^3$. The membrane thus will controll the diffusion if $d/\delta >> 2.10^{-3}$, which will be the case, since $\delta$ = 0.02 cm and the film thickness, d, cannot be greater than the cell itself which is only a few cm wide. In fact, however, the diffusion potential across the enamel membranes did depend on the rate of agitation of the solution. In order to determine whether or not the influence of agitating was important for the results, we measured the emf of a number of cells without and with agitation of the solution. As magnetic stirrers disturb the measurements due to the high impedance of the circuit, the solution was agitated by circulating the solution via a nozzle that is directed against the membranes. As a tube from the cell to the pump and back to the cell acts like a pickup coil when filled with a conducting liquid, the measurements were disturbed by for instance the pump motor which broadens the recorded line to about 2 mV. Therefore the liquid column was interrupted by dropformers before and after the pump. If thse dropformers are made of siliconized glass, the broadening of the line on the recorder is not more than 0.5 mV. The diameter of the nozzles used was 0.3 mm. A pumping rate of about 7 ml $min^{-1}$ had no further effect on the emf, the pumping rate used in the experiments was 10 ml

min$^{-1}$. The distance between nozzle and enamel was 1 to 2 mm.

Fourteen experiments were carried out without and with agitation. With most of the enamel membranes the emf increased when the solution was agitated. On the average this increase was 0.8 mV. The fixed charge calculated for these experiments was, on the average, 0.77 meq l$^{-1}$ greater for the measurements with agitation. The average increase in the calculated ratio in the diffusion coefficients was 0.04. These two values, which are 16 percent and 4 percent of the average values of the fixed charges and the ratio of diffusion coefficients respectively, are statistically significant ($p < 0.01$). If we take into account, however, the standard error in the emf measurements, as calculated from the least squares method (formula 2.4.4), the difference between measurements with and without agitation is negligible. The standard error, which is measure for the fit of the mathematical model to the experimental values was 0.02 mV less without agitation than with. This is much less than the error in the emf readings. The fact that theoretically the film cannot play an important role and that the agitation gave no better results in terms of a least squares fit to the model, made us decide to do the further experiments without agitation of the solution.

### *3.8 Electromotive Force Measurements.*

In the majority of experiments a standard procedure for the measurement of the series of emf's has been used. For such a standard series of measurements seven pairs of electrolyte solutions (mostly RbCl solutions) were used. The concentrations of these solutions are listed in table 4.2.1. For the measurement of the emf one day is needed for each pair of solutions.

The schedule of a day is shown in table 3.8.1.

Table 3.8.1.
Schedule of a day of emf measurements.

| time | action |
|---|---|
| 9.00 | solutions of the previous day refreshed |
| 9.30 - 13.00 | emf recorded |
| 13.00 | electrodes interchanged |
| 13.30 - 17.00 | emf recorded |
| 17.00 | next pair of solutions put into the cell |

The enamel membrane was allowed to equilibrate with each solution during one night. Before the measurements the solution was refreshed. Half way each measurement the electrodes of the cell were interchanged to have a controll on their reliability. At the end of the day a new pair of solutions was put into the cell which could equilibrate during the night. It appeared that if greater jumps in the concentrations of the solution pairs were used, one night is not long enough to get equilibration. The series of solutions were used first in ascending order of concentration and thereafter in descending order. So the standard series of measurements lasts fourteen days of measurement.

Literature Chap. 3.

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Waters N.E., 1970. A cell for membrane and diffusion studies across enamel sections, Archs. Oral Biol. 15, 267-269.

# 4 ELECTROMOTIVE FORCE MEASUREMENTS ON BOVINE DENTAL ENAMEL.

## *4.1 Introduction*

The electrochemical characteristics of dental enamel have been investigated by a number of authors. The first investigators who measured the electromotive force (emf), which develops across dental enamel when it is placed in a concentrationcell, were Amberson, Williams and Klein (1926). Waters (1971, 1972 and 1975) did a great number of experiments with various electrolytes at various pH values, both on human dental enamel and on hydroxylapatite pellets.

These experiments show that enamel behaves like a porous ion selective membrane. As in dental caries the initial lesion is covered by a relatively intact surface layer, it is argued that the transport of ions can be an important factor in the control of the caries process. To investigate the influence of the ionselectivity on the caries process we measured those parameters of enamel that determine this ionselectivity both before (this chapter) and after chemical treatment (chapter 5). Finally, we tried to calculate the influence of the ionselectivity on the caries process (chapter 6).

The materials and methods of our experiments are extensively described in the previous chapter. On the basis of the arguments presented in section 3.7 no distinction is made between measurements done with or without agitation of the solution. When experiments were done with and without agitation, only the results obtained without are given.

## *4.2 Results*

In figure 4.2.1 the measured emf's of a typical experiment

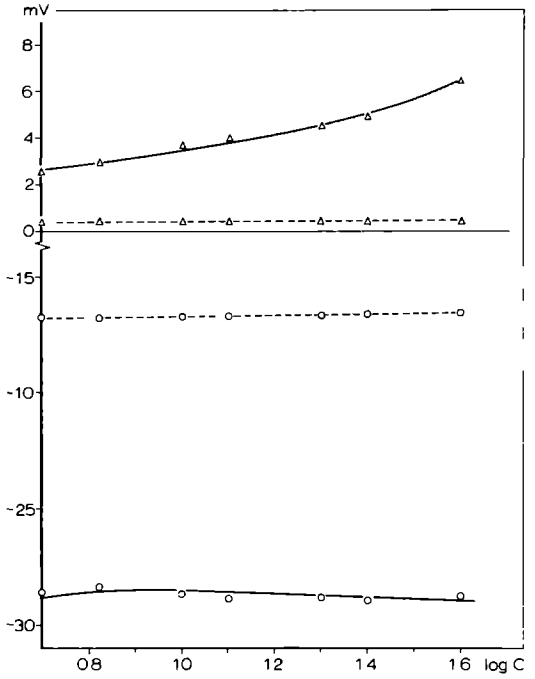


Fig. 4.2.1.

Plots of the measured emf's of experiments ECT2 with RbCl (triangles) and ECT3 with $MgCl_2$ (circles). The solid lines represent the emf's according to the $\omega\bar{X}$ and $\bar{D}_+/\bar{D}_-$ calculated from the measured emf's. The broken lines are the liquid junction potentials.

(ECT 2 and ECT 3) are plotted against the negative logarithm of the concentration in the left compartment. The concentration in the right compartment of the cell is always one fifth of that in the left one. The solid lines are the emf's calculated from the fixed charge and the ratio of the diffusion coefficients as obtained with the program TMS3 (section 2.4 and appendix D). The broken lines represent the liquid junction potentials, which

are the emf's which would have been obtained if the membrane would not have any ionselectivity. They were calculated using the program TMS4 (section 2.2 and appendix B). In Table 4.2.1

Table 4.2.1.

Results of emf measurements on a slice of bovine enamel in a concentration cell. The four consecutive periods last about two or three weeks each (experiment ECT1, 2, 3 and 4).

| concentrations mol $l^{-1}$ | | emf's in mV | | | |
|---|---|---|---|---|---|
| C' | C" | RbCl period 1 | RbCl period 2 | $MgCl_2$ period 3 | RbCl period 4 |
| 0.025 | 0.005 | 5.6 | 6.4 | - 28.8 | 4.1 |
| 0.04 | 0.008 | 6.7 | 4.8 | - 29.0 | 3.7 |
| 0.05 | 0.01 | 4.4 | 4.5 | - 28.8 | 3.9 |
| 0.08 | 0.016 | 5.2 | 4.0 | - 28.9 | 3.6 |
| 0.1 | 0.02 | 4.5 | 3.7 | - 28.7 | 3.1 |
| 0.15 | 0.03 | 3.6 | 2.9 | - 28.4 | 3.1 |
| 0.2 | 0.04 | 2.9 | 2.6 | - 28.6 | 1.2 |

the measurements on enamel section ECT are summarized. They consist of two series of emf measurements with RbCl solutions then a series with $MgCl_2$ solutions and finally a series with RbCl as the electrolyte. In Table 4.2.2 the results of the calculations of these emf's with the program TMS3 are listed. The coefficients b of the formula 2.2.6, which were used were 0.055 for the RbCl solutions and 0.2424 for the $MgCl_2$ solutions. They were obtained from Robinson and Stokes (1970) using a least squares method.

Table 4.2.3.

Results of electrochemical experiments on sections of bovine enamel in RbCl solutions. The figures in parentheses are the calculated standard errors (section 2.4). The length of a period is two or three weeks. The concentration of the fixed charge is expressed in milli equivalents per litre of transport phase.

| | period 1 | | period 2 | | period 3 | | period 4 | |
|---|---|---|---|---|---|---|---|---|
| Exp | $\bar{D}_+/\bar{D}_-$ | $\omega\bar{X}$ | $\bar{D}_+/\bar{D}_-$ | $\omega\bar{X}$ | $\bar{D}_+/\bar{D}_-$ | $\omega\bar{X}$ | $\bar{D}_+/\bar{D}_-$ | $\omega\bar{X}$ |
| ECN | 1.15 (0.02) | - 3.3 (0.4) | 1.29 (0.03) | - 2.5 (0.5) | 1.20 0.07 | 0 (1) | | |
| ECQ | 1.24 (0.02) | - 3.5 (0.4) | 1.62 (0.05) | - 3.0 (0.6) | 1.48 (0.07) | -3 (1) | | |
| ECR | 1.10 (0.07) | - 12 (2 ) | 1.12 (0.01) | - 3.0 (0.3) | | | | |
| ECS | 1.27 (0.09) | - 19 (3 ) | 1.33 (0.02) | -10.9 (0.4) | | | | |
| ECT | 1.18 (0.04) | - 2.0 (0.8) | 1.12 (0.01) | - 2.6 (0.2) | | | | |
| ECU | 1.18 (0.05) | - 2.0 (0.9) | 1.15 (0.02) | - 2.0 (0.5) | | | | |
| ECV | 1.06 (0.02) | + 0.3 (0.5) | 1.07 (0.02) | + 0.1 (0.5) | | | | |
| ECX | 1.17 (0.01) | - 0.4 (0.3) | 1.16 (0.02) | + 0.1 (0.5) | | | | |
| ECAA | 1.19 (0.02) | - 0.3 (0.3) | 1.14 (0.02) | - 0.9 (0.5) | 1.13 (0.01) | -0.3 (0.2) | 1.10 (0.02) | 0.0 (0.4) |
| ECAC | 1.17 (0.01) | + 1.0 (0.2) | 1.13 (0.04) | + 0.1 (0.9) | 1.13 (0.01) | +0.3 (0.2) | 1.10 (0.01) | 0.0 (0.3) |

Table 4.2.2.

Results of the calculations with TMS3 on the data of Table 4.2.1. Figures in parentheses are standard errors (formula 2.4.8 and 2.4.9). $s_E$ is the calculated standard error in the emf's (formula 2.4.4). The values of $D_+/D_-$ in bulk water are from Robinson and Stokes (1970). The concentration of the fixed charge is in milli equivalents per litre of transport phase.

| Exp | electrolyte | $\omega\bar{X}$ | $\bar{D}_+/\bar{D}_-$ enamel | | bulk water | $s_E$ mV |
|---|---|---|---|---|---|---|
| ECT1 | RbCl | -2.0 (0.8) | 1.18 | (0.04) | 1.02 | 0.9 |
| ECT2 | RbCl | -2.6 (0.2) | 1.12 | (0.01) | 1.02 | 0.3 |
| ECT3 | $MgCl_2$ | +1.4 (0.8) | 0.12 | (0.002) | 0.35 | 0.3 |
| ECT4 | RbCl | -1.4 (0.6) | 1.12 | (0.03) | 1.02 | 0.7 |

In Table 4.2.3 the results of the electrochemical measurements on ten sections of bovine enamel are given. These experiments consisted of two or more consecutive series of measurements to obtain information about the time dependence of the properties. The sections ECV and ECX and the sections ECAA and ECAC are halfs of a larger slice of enamel, the other half was treated with some agent. These experiments will be discussed in chapter 5.

The influence of $MgCl_2$ on the selectivity of enamel is given in Table 4.2.4. In the first and the second period the emf measurements are done with RbCl solutions. In the third period $MgCl_2$ was the electrolyte and finally, in the fourth period, RbCl solutions were used. The concentrations of the $MgCl_2$ solutions were the same as those of the RbCl solutions (see Table 4.2.1).

Table 4.2.4.

Results of experiments in which apart from RbCl solutions, $MgCl_2$ solutions have been used. Figures in parentheses are the calculated standard errors (section 2.4). The periods last for two to three weeks. The fixed charge concentration is in milli equivalents per litre of transport phase.

| | period 1 RbCl | | | | period 2 RbCl | | | | period 3 $MgCl_2$ | | | | period 4 RbCl | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Exp | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ | | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ | | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ | | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ | |
| ECR | 1.10 | (0.07) | - 12 | (2) | 1.12 | (0.01) | - 3.0 | (0.3) | 0.20 | (0.03) | + 3 | (4) | | | | |
| ECS | 1.27 | (0.09) | - 19 | (3) | 1.33 | (0.02) | -10.9 | (0.4) | 0.04 | (0.04) | -32 | (7) | 1.29 | (0.02) | + 5.9 | (0.4) |
| ECT | 1.18 | (0.04) | -2.0 | (0.8) | 1.12 | (0.01) | - 2.6 | (0.2) | 0.12 | (0.01) | 1.4 | (0.9) | 1.12 | (0.02) | - 1.4 | (0.6) |
| ECU | 1.18 | (0.05) | -2.0 | (0.9) | 1.15 | (0.02) | - 2.0 | (0.5) | 0.14 | (0.02) | +16 | (6) | 1.16 | (0.03) | - 1.0 | (0.6) |

The last series of experiments which will be described in this chapter are what we called combination experiments. In these experiments two periods of electrochemical measurements are followed by a period of radiochemical measurements. The experiments are concluded with a final series of electrochemical measurements. The radiochemical measurements were carried out with $^{86}Rb^{+}$ and $^{36}Cl^{-}$ as tracers. The total RbCl concentration was 0.01 mol $l^{-1}$ on both sides. The radio-active isotopes were added in one compartment and their concentration was measured in the other compartment of the cell as a function of time. An extensive description of the radiochemical experiments and the necessary calculations is given by Borggeven, Van Dijk and Driessens (1977). The result of this set up is that the selectivity of the enamel membrane is measured by two independent methods. In Table 4.2.5 the results are given of the electrochemical measurements in period 1, 2 and 4 and of the radiochemical measurements of period 3.

As the concentrations of the radiotracers inside the membrane are not known, no true diffusion-coefficients can be calculated from the results of the radiochemical experiments. From the bulk concentrations of the ratio tracers as a function of time and Fick's first line (formula 2.1.4) apparent diffusion coefficients can be calculated which include the effect of the Donnan distribution on the diffusion process. We called the ratio of these apparent diffusion coefficients the selectivity $S_{0.01}$, where the index denotes the concentration of the bulk solutions for which it is measured or calculated. From the electrochemical measurements the Donnan distribution can be calculated for the conditions of the radio-chemical experiments (formula 2.2.10). Together with formula 2.1.4 we then can calculate the ratio of the apparent diffusion coefficients $S_{0.01}$:

Table 4.2.5.

Results of two combined electrochemical and radiochemical experiments. The radiochemically obtained apparent diffusion coefficients are expressed in $10^{-8} cm^2 s^{-1}$, the fixed charge in milli equivalents per litre of transport phase. Figures in parentheses are the calculated standard errors (section 2.4). Each period lasts about two to three weeks.

| | period 1 | | | | period 2 | | | | period 3 | | | | period 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Exp | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ | | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ | | $\bar{D}^*_{Rb^+}$ | | $\bar{D}^*_{Cl^-}$ | | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ | |
| ECO | 1.24 | (0.03) | -3.0 | (0.6) | 1.45 | (0.04) | -1.2 | (0.5) | 48 | (2) | 35 | (2) | 1.23 | (0.02) | -4.3 | (0.5) |
| ECP | 1.03 | (0.03) | -6.0 | (0.9) | 1.26 | (0.05) | -2.2 | (0.8) | 3.63 | (0.06) | 2.28 | (0.07) | 1.66 | (0.06) | -6 | (1) |

$$S_{0.01} = \frac{\bar{D}_+}{\bar{D}_-} r^2_{0.01} \qquad 4.2.1$$

The use of this formula enables a comparison between the results of the electrochemical and radiochemical experiments.

In table 4.2.6 the selectivities of the four enamel sections are tabulated. The values for the first, second and last

Table 4.2.6.

Selectivity of enamel sections calculated from the data of Table 4.2.5. The figures in parentheses are standard errors, calculated from the standard errors in the parameters of Table 4.2.5.

| Exp | period 1 $S_{0.01}$ | period 2 $S_{0.01}$ | period 3 $S_{0.01}$ | period 4 $S_{0.01}$ |
|---|---|---|---|---|
| ECO | 1.7 (0.1) | 1.6 (0.1) | 1.4 (0.1) | 1.9 (0.1) |
| ECP | 1.9 (0.2) | 1.6 (0.1) | 1.6 (0.1) | 3.0 (0.3) |

period were calculated from $\bar{D}_+/\bar{D}_-$ and $\omega\bar{X}$ using 2.2.19 and 4.2.1. Those of the third period from the two apparent diffusion coefficients obtained with the radiochemical experiments.

*4.3 Discussion.*

Since the measurements of Amberson, Williams and Klein (1926) it has been known that dental enamel behaves like an ionselective membrane. From their measurements it could be concluded that in KCl solutions the charge of the enamel is negative and in $CaCl_2$ solutions it is positive. The important conclusion from their work is that transport of ions is possible

through dental enamel and that the transport properties of enamel depend on its electrolytic environment.

The measurements of Waters (1971, 1972 and 1975) confirm the results of Amberson et al. (1926). The electrolytes used by Waters were KCl, NaCl, $CaCl_2$, $KH_2PO_4$ and $K_2HPO_4$. The membranes he used were enamel caps of whole human teeth, sections of human dental enamel and pellets of synthetic hydroxylapatite. From these measurements it could be concluded that with the exception of $CaCl_2$, enamel is cationselective in all electrolytes. The ionselectivity was larger in the phosphate solutions than in the chloride solutions. The change in ionselectivity induced by $CaCl_2$ disappears when the $CaCl_2$ solutions were replaced by a KCl solution. A full discussion of the measurements of Waters and their evaluation with the program TMS3 is given by Van Dijk et al. (1977).

The technique of our emf measurements differs on a few points from that of Waters. Firstly we used Ag/AgCl electrodes instead of calomel half cells with salt bridges (section 3.3) and we did not agitate the solution (section 3.7). Secondly we used solutions of which the concentration in the right compartment was always one fifth of that in the left one, while Waters kept the concentration in the left one constant at 0.1 mol $l^{-1}$. Our method had the advantage that a constant gradient in the logarithm of the concentration eliminates for a part the necessity of a correction for activity coefficients. Also we hoped that the choice of a constant ratio of the concentrations would improve the accuracy with which the fixed charge could be determined. For, if there is no fixed charge the potential will not significantly depend on the concentration if the ratio of the two concentrations is constant. Model calcula-

tions, however, showed that this latter advantage is not important.

Figure 2.4.1 shows that there is a good agreement between measured and calculated emf's for both experiments. We accepted as a measure for the fit of the experimental data to the model, represented by the formulas of section 2.2, the square root of the sum of squares of the difference between measured and calculated emf's, divided by the number of degrees of freedom (formula 2.4.4). In Table 4.3.1 these calculated standard errors are listed. The average of these twentysix values is 0.6 mV. This value is acceptable but perhaps a bit larger than can be explained by the experimental error.

Table 4.3.1.
Calculated standard errors in the potential in mV of the experiments mentioned in Table 4.2.3.

| Exp | period 1 | period 2 | period 3 | period 4 |
|---|---|---|---|---|
| ECN | 0.48 | 0.63 | 1.60 | |
| ECQ | 0.43 | 0.73 | 1.15 | |
| ECR | 1.54 | 0.29 | | |
| ECS | 1.26 | 0.28 | | |
| ECT | 0.91 | 0.25 | | |
| ECU | 1.08 | 0.56 | | |
| ECV | 0.60 | 0.60 | | |
| ECX | 0.32 | 0.58 | | |
| ECAA | 0.41 | 0.58 | 0.22 | 0.44 |
| ECAC | 0.20 | 1.01 | 0.23 | 0.28 |

Up to now some 70 sections of bovine enamel have been investigated electrochemically. The average value of the fixed charge (including its sign) of the sections which were not in contact with other solutions than the RbCl solutions is about -2 meq $l^{-1}$. The average value of the ratio of the diffusion coefficients of these sections is about 1.2. The resulting selectivity, $S_{0.01}$ is 1.5 on the average. The general conclusion from all observations made during these experiments is that enamel tends to behave irregularly. Examples of what could be called abnormal sections are ECR and ECS. They show a high negative fixed charge in the first period and a more normal one in the second period (Table 4.2.3). The fact that the charge changes during these series of measurements causes the large errors in the calculated parameters because in the model it is assumed that these parameters are constants. Up to now half a dozen sections showed this abnormal behaviour.

It is quite probable that the high standard errors in the emf's in other experiments are for the major part the cause of some unexpected behaviour of the enamel. Another aspect of this behaviour is the fact that after an adaptation period the emf of a cell very slowly decreases by one or two millivolts a day. After the solutions are refreshed the emf starts again on the same value as the day before. From the diffusion coefficients measured radiochemically by Borggreven, Van Dijk and Driessens (1977) it can be calculated that the decrease in potential cannot be explained by the decrease of the concentration gradient due to diffusion. After refreshing the solutions it takes some time before the emf stabilizes. This adaptation period of up to one or two hours is much longer than is expected on the basis of computer simulations (using a simplified version of the dynamic caries model; see chapter 6, appendix F). The adaptation periods

are partially caused by the electrodes and by another part by the membrane. If the enamel is replaced by some synthetic membrane these periods are much shorter. From these observations it is obvious that the solutions used were not in equilibrium with the enamel sections in spite of the equilibration period (sections 3.1 and 3.6). In what sense they deviate from equilibrium is not clear.

The results of the measurements on bovine enamel, which are tabulated in the Tables 4.2.1 through 4.2.4, are in agreement with the observations of Waters (Van Dijk et al.1977) on human enamel. In KCl solutions the fixed charge of human enamel is more negative than that of bovine enamel ($\omega\bar{X} \simeq -80$ meq $l^{-1}$) resulting in a higher ion selectivity ($S_{0.01} = 8$ on the average). Solutions of $MgCl_2$ behave in the same way as the $CaCl_2$ solutions used by Waters. They both reduce the negative fixed charge or sometimes change it from negative into positive. As magnesium is not readily incorporated in the apatite lattice this might indicate that the effect of both the $CaCl_2$ and tne $MgCl_2$ solutions is restricted to the double layer on the walls of the pores of enamel. This is supported by radiochemical experiments in which the behaviour of radioactive $^{45}Ca^{++}$ is studied as a function of the $Ca^{++}$, $Mg^{++}$ or $Rb^{+}$ concentration and of time (Borggreven, Van Dijk and Driessens, 1977). If an enamel membrane is equilibrated in a concentrationcell with $^{45}Ca^{++}$ present in one of the compartments, the addition of both non-radioactive $CaCl_2$ and of $MgCl_2$ induced a rapid outflow of previously adsorbed $^{45}Ca^{++}$ whereas an addition of RbCl to the same ionic strength did not.

The combined electrochemical and radiochemical experiments (Table 4.2.5 and 4.2.6) show that the ionselectivities,calculated from the results of both methods are comparable. Both

methods have their advantages. The electrochemical experiments do not have the disadvantages of the handling of radioactive compounds. They yield information about the concentration dependence of the transport of ions through dental enamel, whereas the radiochemical experiments yield values at only one concentration by measuring over the same period of time. The radiochemical experiments however yield absolute values for the diffusion coefficients, the electrochemical ones only ratios of them. Another advantage of the radiochemical method is that the diffusion of uncharged components can be studied as well.

Although the Teorell Meyer and Sievers theory does fit fairly well to the experimental data it is necessary to make some remarks about the physicochemical meaning of the transport parameters. In the original Teorell Meyer and Sievers theory (Teorell, 1954) the fixed charge is defined as the excess of fixed ionized groups with either a positive or a negative charge on the walls of the pores, in equivalents per unit of pore volume. This is equivalent with the ion exchange capacity. The theory does not explain why the ratio of the diffusion coefficients within the membrane should be different from that in bulk water. Certainly not for those cases in which the ion selectivity is not influenced by factors of pore dimensions and ionic radii (House, 1974). The fact that in most enamel specimens this ratio differs significantly from that in bulk water (Tables 4.2.2, 4.2.3 and 4.2.4) means that the pore walls or the organic matrix of enamel have some extra interaction with the moving ions. A possible explanation can be that the fixed charge reduces the effective pore volume available for the transport of coions (ions having the same sign of charge as the fixed charge) and not that of the counter-ions.

Such interaction is not accounted for in the Teorell Meyer and Sievers theory. Thus the physicochemical meaning of the fixed charge as calculated from our measurements is not strictly the same as that in this theory. In the present study the fixed charge must merely be seen as a transport parameter which represents the concentration of the ion selectivity.

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# 5 ELECTROMOTIVE FORCE MEASUREMENTS ON TREATED BOVINE DENTAL ENAMEL.

## *5.1 Introduction.*

The influence of a treatment with certain chemical agents on the ion selectivity of dental enamel and synthetic hydroxylapatite has been investigated by Tung (1976) and Waters (1972).

Waters treated human dental enamel with a 1 mol $l^{-1}$ KF solution and found an increase of the ionselectivity with about a factor two. Tung treated synthetic hydroxylapatite with phytate solutions and with solutions that contained protamine. The phytate solutions increased the cation selectivity while protamine made the membranes anion selective.

In this chapter the treatment of sections of bovine enamel with a number of agents and their effect on the ionselectivity will be discussed. Most of the compounds used were mentioned in the literature as potentially anticariogenic. We used orthophosphate, pyrophosphate, tri-metaphosphate and hexametaphosphate (McGaughey and Stowell, 1977). Also used were phytate (Magrill, 1973), 1,1 ethylhydroxyldiphosphonate (EHDP) (Wölltgens, 1975), fluoride and monofluorophosphate (Feller et al., 1976).

## *5.2 Materials and Methods.*

All experiments described in this chapter had the following setup:

1. characterization of the enamel by a series of emf measurement before treatment. The electrolyte used was RbCl in the concentrations mentioned in Table 4.2.1;

2. treatment on both sides of the section of enamel with a solution of the agent to be tested, during four hours;
3. characterization of the enamel by another series of emf mesurements with RbCl solutions.

A measuring period (steps 1 and 3) lasts two to three weeks (section 3.8).

The concentration of each of the tested compounds was 0.1 mol $l^{-1}$ except for that of the EHDP solution which was 0.02 mol $l^{-1}$ due to its limited solubility at pH = 7.4. The pH of the solutions was adjusted to pH = 7.4 with hydrochloride acid or potassium hydroxyde.

In addition to this type of experiment, phytate and fluoride were studied more extensively. For these compounds the influence of the concentration and the duration of the treatment were investigated. In these experiments the selectivity was not always calculated from a series of emf measurements as in the above mentioned ones but from the measurements at a single concentration. By this method the selectivity can be estimated as a function of time. The solutions used for these so called one-point measurements were 0.025 and 0.005 mol $l^{-1}$ RbCl.

From these one-point measurements neither the fixed charge nor the ratio of the diffusion coefficients can be calculated. To make the results of these experiments comparable with those of the other experiments a parameter is calculated which will have a meaning not far from the selectivity, $S_{0.01}$, introduced in section 4.2 (formula 4.2.1). We shall call this parameter also selectivity (denoted by $\tilde{S}$). It is defined as the ratio of the diffusion coefficients which is obtained if the formula of Nernts (Laksminarayanaiah, 1969) is used. On rearrangements

this formula becomes

$$\tilde{S} = \frac{\frac{RT}{F} \ln \frac{C'}{C''} + E}{\frac{RT}{F} \ln \frac{C'}{C''} - E} \qquad 5.2.1$$

Table 5.2.1.

Comparison of the selectivities calculated from emf measurements with the formula 4.2.1 (S) and the formula 5.2.1 ($\tilde{S}$).

| | period 1 | | period 2 | | period 3 | | period 4 | |
|---|---|---|---|---|---|---|---|---|
| Exp | S | $\tilde{S}$ | S | $\tilde{S}$ | S | $\tilde{S}$ | S | $\tilde{S}$ |
| ECV | 1.02 | 1.01 | 1.06 | 1.02 | 0.98 | 1.00 | | |
| ECW | 1.12 | 1.09 | 3.78 | 2.93 | 7.17 | 4.43 | 4.59 | 3.46 |
| ECX | 1.22 | 1.18 | 1.15 | 1.12 | 1.23 | 1.13 | | |
| ECY | 1.19 | 1.13 | 3.24 | 2.60 | 7.89 | 4.65 | 4.83 | 3.53 |

In Table 5.2.1 the selectivities S and $\tilde{S}$ are listed, which were calculated with the formulas 4.2.1 from a series of experiments and with formula 5.2.1 from the one point measurements respectively.

In Table 5.2.2 the codes of the sections, the compounds with which they were treated and the concentrations used, are listed. The experiments ECAE through ECAJ are the one point measurements mentioned above. All other experiments are of the setup described in the first paragraph of this section.

The purity of the chemicals used was always analytical grade or the highest purity commercially available. They were not subjected to further purification in our laboratory.

Table 5.2.2.
Codes for the enamel sections and the chemical agents with their concentrations.

| name of enamel sections | name of agent | concentrations mol $l^{-1}$ |
|---|---|---|
| EVC ECX | ortophosphate | 0.1 |
| ECAO ECAP | pyrophosphate | 0.1 |
| ECAM ECAN | trimetaphosphate | 0.1 |
| ECAK ECAL ECAX ECAZ | hexametaphosphate | 0.1 |
| ECW ECY ECAE through ECAJ | phytate | 0.01 0.03 0.1 |
| ECAB ECAD ECAW ECAY | EHDP | 0.02 |
| ECAQ ECAR ECAT ECAV | monofluorophosphate | 0.1 |
| ECAS ECAK | fluoride | 0.1 |

## *5.3 Results.*

### *5.3.1 Phytate Treatment.*

In Table 5.3.1 the results are listed of the calculations with the program TMS 3 (section 2.4 and appendix C) on the

Table 5.3.2.
The selectivity of bovine enamel before and after treatment with phytate. S% is the selectivity in percentages relative to the original selectivity. The results in period 5 are obtained from radiochemical experiments. Treatments with 0.01 mol $l^{-1}$ solution and 0.1 mol $l^{-1}$ solution between periods 1 and 2 and between periods 2 and 3 respectively.

| Exp | period 1 | | period 2 | | period 3 | | period 4 | | period 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $S_{0.01}$ | S% | $S_{0.01}$ | S% | $S_{0.01}$ | S% | $S_{0.01}$ | S% | $S_{0.01}$ | S% |
| ECV | 1.02 | 100 | 1.06 | 104 | 0.98 | 96 | | | | |
| ECW | 1.12 | 100 | 3.78 | 338 | 7.17 | 640 | 4.59 | 410 | 3.72 | 340 |
| ECX | 1.22 | 100 | 1.15 | 94 | 1.23 | 101 | | | | |
| ECY | 1.19 | 100 | 3.24 | 272 | 7.89 | 663 | 4.83 | 406 | 3.86 | 324 |

Table 5.3.1.

Transport properties of bovine enamel before and after treatment with phytate solutions. Treatment between period 1 and 2 with a 0.01 mol $l^{-1}$ solution and between period 2 and 3 with a 1 mol $l^{-1}$ solution. ECV and ECX are untreated duplicate sections. The fixed charge, $\omega\bar{X}$, is in milli equivalents per litre, $\bar{D}_+/\bar{D}_-$ is the ratio of the diffusion coefficients of $Rb^+$ and $Cl^-$.

| Exp | period 1 | | | period 2 | | | period 3 | | | period 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ | $\bar{D}_+/\bar{D}_-$ | | $\omega\bar{X}$ |
| ECV | 1.06 | (0.02) | 0.3 (0.5) | 1.07 | (0.02) | 0.1 (0.5) | 1.09 | (0.04) | 1.0 (1.09) | | | |
| ECW | 1.06 | (0.01) | -0.5 (0.3) | 2.05 | (0.07) | -6.2 (0.9) | 2.4 | (0.2) | -12 (3) | 2.41 | (0.09) | -7 (1) |
| ECX | 1.17 | (0.01) | -0.4 (0.2) | 1.16 | (0.02) | 0.1 (0.5) | 1.12 | (0.05) | -1 (2) | | | |
| ECY | 1.17 | (0.03) | -0.2 (0.6) | 1.95 | (0.07) | -5 (1) | 2.6 | (0.2) | -12 (3) | 2.38 | (0.09) | -7 (1) |

measured emf's before and after treatment with phytate. ECV and ECW as well as ECX and ECY were pairs of duplicate sections obtained from two larger sections of enamel. The sections ECW and ECY were both treated after period 1 and period 2; the other two sections were not treated.

In Table 5.3.2 the selectivities in a 0.01 mol $l^{-1}$ RbCl solution are listed together with the relative increase in percentages with respect to the first period. The selectivity $S_{0.01}$ was calculated with formula 4.3.1. The results in period 5 are obtained from radiochemical experiments by Borggreven, Van Dijk and Driessens (1977).

In Table 5.3.3 the influence of the concentration and the duration of treatment are shown. The sections ECAE and ECAF, ECAG and ECAH and the sections ECAI and ECAJ were pairs of duplicate sections. Only the relative increase in percentages of the selectivity as calculated from the emf with the formula 5.2.1 are displayed. The measurements were done 24 hours after the completion of the treatment.

Table 5.3.3.
The selectivity (formula 5.2.1) of bovine enamel as a function of the concentration of the phytate solution and the duration of each successive treatment. The selectivity is in percentages of the original selectivity. Concentrations in mol $l^{-1}$ and times in hours.

| Exp | phytate concentration | duration of treatment 0.5 | 1.5 | 4.5 | 68 | 92 |
|---|---|---|---|---|---|---|
| ECAE | 0.01 | 135 | 151 | 230 | 382 | 454 |
| ECAF | 0.01 | 126 | 144 | 186 | 339 | 388 |
| ECAG | 0.03 | 290 | 385 | 471 | 708 | 765 |
| ECAH | 0.03 | 270 | 365 | 432 | 634 | 644 |
| ECAI | 0.1 | 341 | 404 | 546 | 706 | 776 |
| ECAJ | 0.1 | 500 | 739 | 682 | 790 | 671 |

After the enamel sections were treated during 68 hours the selectivity was measured as a function of the time elapsed after this treatment was completed. The results of these experiments are listed in Table 5.3.4.

Table 5.3.4.

The selectivity (formula 5.2.1) after treatment of bovine enamel during 68 hours with phytate solutions, in percentages of the original selectivity, as a function of the time elapsed after the finish of the treatment. Phytate concentrations in mol $l^{-1}$, time in hours.

| Exp | phytate concentration | time elapsed after treatment | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 3 | 24 | 48 | 72 |
| ECAE | 0.01 | 547 | 500 | 382 | 309 | 276 |
| ECAF | 0.01 | 445 | 400 | 339 | 276 | 255 |
| ECAG | 0.03 | 614 | 720 | 708 | 561 | 540 |
| ECAH | 0.03 | 614 | 673 | 634 | 535 | 521 |
| ECAI | 0.1 | 695 | 755 | 706 | 635 | 677 |
| ECAJ | 0.1 | 740 | 866 | 790 | 694 | 717 |

*5.3.2 Treatment with Some Inorganic Phosphates.*

A treatment of bovine enamel with a 0.1 mol $l^{-1}$ orthophosphate solution during four hours had no measurable influence on its ionselectivity. The other phosphates had an influence on the the ionselectivity which increased with increasing molecular weight. The results of treatments with 0.1 mol $l^{-1}$ solutions of pyrophosphate, trimetaphosphate and hexametaphosphate are listed in the Table 5.3.5. They are expressed in percentages of the ionselectivity before treatment and were calculated with formula 4.2.1.

Table 5.3.5.

Selectivity of bovine enamel in percentages of the original selectivity after treatment with various phosphates. The concentrations of the solutions were 0.1 mol $l^{-1}$ and the treatments lasted four hours.

| Exp | compound | period 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| ECAO | pyrophosphate | 100 | 150 | 115 | |
| ECAP | pyrophosphate | 100 | 142 | 109 | |
| ECAM | trimetaphosphate | 100 | 294 | 187 | |
| ECAN | trimetaphosphate | 100 | 179 | 123 | |
| ECAK | hexametaphosphate | 100 | 508 | 264 | |
| ECAL | hexametaphosphate | 100 | 447 | 173 | |
| ECAX | hexametaphosphate | 100 | 290 | 221 | 194 |
| ECAZ | hexametaphosphate | 100 | 330 | 245 | 186 |

Table 5.3.6.

Selectivity of bovine enamel expressed in percentage of the original selectivity after treatment with fluoride. Treatment time in hours and concentration in mol $l^{-1}$.

| Exp | treatment time | conc. | period 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|
| ECAS | 4 | 0.1 | 100 | 107 | 128 | 162 |
| ECAU | 4 | 0.1 | 100 | 110 | 114 | 134 |
| ECBA | 4 | 0.025 | 100 | 112 | 112 | |
| ECBB | 4 | 0.1 | 100 | 101 | 104 | |
| ECBC | 4 | 0.025 | 100 | 114 | 114 | |
| ECBD | 4 | 0.1 | 100 | 111 | 113 | |
| ECBI | 4 | 0.01 | 100 | 129 | 129 | |
| ECBJ | 4 | 0.1 | 100 | 120 | 123 | |
| ECBK | 16 | 0.01 | 100 | 109 | 135 | |
| ECBL | 16 | 0.1 | 100 | 125 | 105 | 157 |
| ECBM | 64 | 0.01 | 100 | 123 | 139 | |
| ECBN | 64 | 0.1 | 100 | 108 | 108 | 119 |

*5.3.3 Treatment with Fluoride and Monofluorophosphate (MFP).*

The treatments with fluoride were done with solutions of RbF of 0.01, 0.025 or 0.1 mol $l^{-1}$ at its natural pH of 7.6. In Table 5.3.6 the results are listed. The selectivities, calculated with formula 4.2.1, are expressed in percentages of the selectivity before treatment. Before period 2 a series of one-point measurements with 0.025 - 0.005 mol $l^{-1}$ RbCl solutions were carried out to investigate the time dependence of the ion selectivity after treatment. Contrary to the other compounds tested the selectivity tends to increase during this period. In figure 5.3.1 this time dependence of the selectivity (calculated with formula 5.2.1) is shown for experiment ECAU and is representative for all experiments.

In Table 5.3.7 the results of a treatment with MFP are listed. The sections ECAT and ECAV are duplicate sections of ECAS and ECAU respectively, which were treated with RbF solutions. This enables a comparison of the results of a fluoride and a monofluorophosphate treatment.

*5.3.4 Treatment with 1.1 ethylhydroxydiphosphonate (EHDP).*

The treatment with EHDP was done with a 0.02 mol $l^{-1}$ solution of pH = 7.4 during four hours. Solutions of higher concentration tend to become milky. In Table 5.3.8 the results of the treatments are listed. The selectivities, calculated with formula 4.2.1 are expressed in percentages of the selectivity before treatment.

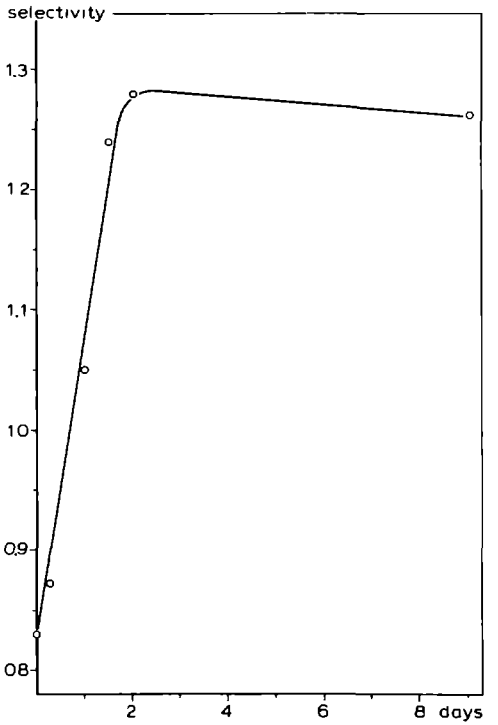


Fig. 5.3.1.

The selectivity of enamel section ECAU (calculated with formula 5.2.1) as a function of time (in days) starting immediately after the finish of the treatment. The dotted line indicates the selectivity before treatment.

Table 5.3.7.

The selectivity of bovine enamel after treatment with MFP during four hours expressed in percentages of the selectivity before treatment. Concentration of the MFP solution was 0.1 mol $l^{-1}$.

| Exp | period 1 | period 2 | period 3 | period 4 |
|---|---|---|---|---|
| ECAQ | 100 | 533 | 226 | |
| ECAR | 100 | 428 | 220 | |
| ECAT | 100 | 234 | 208 | 152 |
| ECAV | 100 | 228 | 219 | 165 |

Table 5.3.8.

Selectivity of bovine enamel after treatment with EHDP during four hours , expressed in percentages of the selectivity before treatment. The concentration of the EHDP solution was 0.02 mol $l^{-1}$.

| Exp | period 1 | period 2 | period 3 | period 4 |
|---|---|---|---|---|
| ECAB | 100 | 286 | 214 | |
| ECAD | 100 | 245 | 188 | |
| ECAW | 100 | 290 | 196 | 234 |
| ECAY | 100 | 300 | 275 | 260 |

## 5.4 DISCUSSION.

### *5.4.1 Phytate Treatment.*

The results of the experiments in which sections of bovine dental enamel were treated with phytate solutions clearly show that these solutions have a significant influence on the selectivity of enamel (Table 5.3.1). Both the ratio of the diffusion coefficient and the absolute value of the fixed charge increase considerably (see also section 4.3, last paragraph). The resulting selectivity (formula 4.2.1) can reach values of seven or more.

Table 5.3.3 shows that the selectivity increases both with an increase in the concentration of the phytate solution and with the duration of the treatment. An increase of the ionselectivity to a certain value is obtained in a shorter time with a solution of higher concentration, which can be explained by the higher rate of diffusion due to the larger concentration gradient. From Table 5.3.2 and 5.3.4 it can be seen that the change

in selectivity induced by phytate solutions is rather persistent. The treatment with a solution of higher concentration seems to be more persistent than that with a lower one. This cannot be explained by diffusion because the time constants of diffusion processes in dental enamel are very much shorter than those of the release processes (see also section 6.5).

The figures of Table 5.3.2 may suggest that the time necessary to let the selectivity decrease to half its maximum value is at least one month. It should be realised in this context that the RbCl solutions in the cells are refreshed twice a day. This means that for each measuring period the section is rinsed at least fourteen times with 18 ml of a RbCl solution at each side of it. The fact that the selectivity as calculated with formula 5.2.1 in Table 5.3.4 is lower directly after the treatment than three hours later might be due to adaptation phenomena and have no physical meaning.

It has been reported that phytate decreases the solubility (Magrill, 1973) and the dissolution rate (Brady, Napper , Smythe, 1966) of hydroxylapatite. This in combination with the results of our experiments show that, potentially, phytate is effective and persistent enough to be used successfully as an anticariogenic agent in tooth pastes, mouth washes or application gels.

### 5.4.2 *Treatment with some Inorganic Phosphates.*

Table 5.3.5 clearly shows that a treatment of bovine enamel with inorganic phosphates is more effective in terms of ionselectivity with increasing molecular weight of the phosphate, i.e. with increasing number of phosphate groups per molecule. The comparison of the selectivity in the first period and the subsequent periods suggests that the persistence of the treatment

also increases with the increasing molecular weight. Orthophosphate increases the ionselectivity, which can be deduced from the observations of Waters (Van Dijk et al., 1977) but a treatment with orthophosphate has no persistency, pyrophosphate has little effect and hexametaphosphate is both the most persistent and effective compound of these four phosphates.

The results suggest that if these compounds are anticariogenic (McGaughey and Stowell, 1977) especially hexamethaphosphate is both effective and persistent enough to be potentially successful in a prophylactic product.

*5.4.3 Treatment with Fluoride and Monofluorophosphate (MFP).*

The treatment of bovine enamel with fluoride seems to have less effect on the ionselectivity than could be expected from the results of the measurements of Waters (Van Dijk et al., 1977). As can be seen from Table 5.3.6 we could not obtain a doubling of the selectivity. After a fluoride treatments the selectivity, however, rises gradually. From figure 5.3.1 it can be seen that directly after the fluoride treatment the enamel is slightly positively charged. This suggests that initially a layer of $CaF_2$ is formed on the surface of the apatite crystals, as Brunetti and Brown (1976) found, that $CaF_2$ is positively charged. The gradual increase of the fixed charge can then be explained by a gradual release of the $CaF_2$ and a reaction with the enamel to a fluoride containing apatite.

From Table 5.3.7 it is obvious that the behaviour of MFP is entirely different from that of fluoride and also from that of orthophosphate. The difference with fluoride can best be deduced from the results of the sections ECAS for fluoride and ECAT for MFP which are duplicate sections made from one larger

section. The same applies for the sections ECAU and ECAV. It is not probable that the large increase of the ion selectivity caused by a MFP treatment is caused by impurities in the form of condensed phosphates.
This because the most abundant impurity in MFP is reported to be pyrophosphate (Ingram, 1977) and this has on its own, a much smaller, effect on the selectivity than MFP has. There is no evidence that the effect of MFP is in fact a combined effect of fluoride and phosphate, obtained from the MFP by hydrolysis in situ. This hydrolysis becomes important at lower pH values (Eanes, 1976) and it has been proposed that the $PO_4F^{2-}$ ion exchanges at the enamel surface for $HPO_4^{2-}$ before hydrolysis (Duff, 1973).

The persistence of the effect of MFP on the ionselectivity together with its effect on the rate of dissolution of enamel apatite (Feller, 1976) indicate that it can be successful as an anticariogenic agent in profilactic products.

The results of the experiments with fluoride indicate that its known effect on the rate of the caries process will not be caused by an effect on the ionselectivity of the enamel but merely on its effect on the solubility and on the rate of dissolution of hydroxylapatite (Huffman et al., 1957; Catress, 1972).

*5.4.4 Treatment with 1,1 ethylhydroxydiphosphonate (EHDP).*

From the data presented in Table 5.3.8 it can be seen that the effect of an EHDP treatment on the ionselectivity of bovine enamel is considerable. Although the treatment was done with a solution of one fifth of the concentration used for the condensed phosphates (sections 5.3.2, 5.4.2) the effect is of comparable magnitude of that with hexametaphosphate despite the larger

number of basic groups than in EHDP. The effectiveness of EHDP as compared to that of hexametaphosphate is well demonstrated by the experiments with the sections ECAV and ECAX, which were duplicate sections made from one larger section as were ECAY and ECAZ. The comparison of the results (Table 5.3.5 and 5.3.8) show that the effect of both compounds is initially the same but that of EHDP has a greater persistency. The persistency of the effect of EHDP on the selectivity together with its effect on the rate of dissolution of calciumphosphates like apatite make it a potentially anticariogenic additive for profilactic products (Wöltgens, 1975).

### *5.4.5 General Discussion and Conclusions.*

The results presented in this chapter indicate that the transport properties of dental enamel can undergo considerable changes as the result of a treatment with various phosphate containing compounds. The effect on the ionselectivity and its persistence increases with an increasing number of phosphate groups per molecule. Two compounds form remarkable exceptions. The effect of EHPD is both stronger and more persistent than that of the comparable pyrophosphate. The effect of MFP is remarkable in two ways. It is more persistent than that of ortophosphate and it seems stronger than that of fluoride. In addition, the time dependence of the ion selectivity induced by MFP is entirely different from that induced by fluoride.

As a consequence of the electroneutrality condition an increase of the ion selectivity will decrease the rate of longitudinal transport of both anions and cations. The computer simulations discussed in chapter 6, however, indicate that this effect will not be very significant for the rate of the caries

process.

It is quite well possible that compounds like the condensed phosphates or organic phosphates and phosphonates exhibit special interactions with for instance the $Ca^{++}$ ion and thus have an entirely different effect on the longitudinal transport of these special ions than it has on the rubidium and chloride ions used in the measurements. The combination of measurements on the enamel solubility and dissolution rate before and after treatment with potentially anticariogenic compounds together with electrochenical measurements of the type discussed in this chapter give, however, valuable information of the effectiveness of the compounds investigated.

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# 6 A MATHEMATICAL DESCRIPTION OF CARIES.

## *6.1 Introduction.*

The use of a mathematical model of a process can be two-fold. In the first place working with the model can improve the understanding of the real process. In the second place the model can be used to simulate the result of affecting the process.

In the case of the caries process one is most interested in the question which factors determine the rate of the demineralization. If a mathematical model is available, all relevant factors can be tested in this respect. For instance, a caries simulation model might answer the question if the transport parameters like ionselectivity, which have been discussed in the previous chapters, have a significant effect on the caries process. The combination of measuring the parameters, that appear to be important for the rate of the caries process, before and after some treatment, and the simulation of the process, can give valuable information about the possible anticariogenicity of that treatment. This can reduce the need of expensive and time consuming animal experiments.

The system in which caries develops can roughly be divided into four compartments:

1. the oral cavity including saliva;
2. the plaque and pellicle;
3. the enamel;
4. the dentine and pulp.

The mathematical models discussed in this study are restricted to physicochemical processes that occur in the third compartment, the enamel. They eventually include a diffusion film on the surface.

Various attempts have been made to describe the caries process in mathematical terms. The most important of these are the one of Holly and Gray (1968) and of Zimmerman (1966a, b, c). Both models and the model presented in this chapter are primarily meant to describe the in vitro caries process (chapter 7).

In the model of Holly and Gray it is assumed that all processes are fast as compared to the longitudinal diffusion of undissociated acid through the pores of enamel. The ratio of the diffusion coefficients in the presupposed, relatively intact, surface layer and in the body of the lesion is assumed to be equal to the ratio of the fractional pore cross section of these two regions. The conclusion of their theory is that the total amount of acid used during demineralization is a non-linear function of the time with four parameters. These are the two fractional cross sections of the pores, the thickness of the superficial layer and the diffusion coefficient of the undissociated acid per unit of pore cross section.

The model of Zimmerman (1966c) is a more fundamental one. No special presuppositions concerning the mechanism of lesion formation, like the formation of a second solid phase or viscosity of the plaque, are made. The more general physicochemical basis of his model involves the diffusion of all of the most important ions like calcium, various phosphate ions and both the undissociated and dissociated acid. The solution in the pores is assumed to be in equilibrium with the apatite, which means that in the model of Zimmerman the dissolution reaction is assumed to be fast as compared to the diffusion processes. The mathematical elaboration of his model is mainly an analytical one. This, in combination with the limitations of the computers in 1966 limited the possibilities of his computer simulations.

The mathematical model for the caries process to be described in this chapter is more elaborate than the two models mentioned above. Our model includes apart from longitudinal diffusion the dissolution reaction of the mineral, neither of which is presupposed to be faster than the other. It includes additional components and equilibria.

## *6.2 The Physicochemical Processes During Caries.*

As far as the enamel is concerned, caries is considered to be a complex process consisting of three basic types of physicochemical processes:

1. diffusion of ions and molecules through enamel pores;
2. dissolution and/or recrystallization of mineral;
3. complexation of the various ions which play a role in the caries process.

ad 1.
The flux of a species i is described by its Nernst-Planck flux equation:

$$J_i = - \bar{D}_i \left( \frac{d\bar{C}_i}{dx} + \bar{C}_i \frac{d \ln \bar{f}_i}{dx} + Z_i \frac{F}{RT} \bar{C}_i \frac{d\bar{E}}{dx} \right) \qquad 2.1.5$$

The rate at which the concentration of i changes due to diffusion is given by

$$\frac{d\bar{C}_i}{dt} = - \frac{1}{\alpha} \frac{dJ_i}{dx} \qquad 6.2.1$$

The effective fractional pore volume, $\alpha$, is introduced because the flux is referred to a unit surface of enamel but the concentration to a unit volume of pore solution. The physical constants

R and F, the temperature and the charge of the species are assumed to be constant. All other parameters in these equations may be a function of the distance to the enamel surface, of time and of each other. The diffusion coefficient for instance is assumed to be a function of the effective fractional pore volume which is on its turn a function of time and distance.

ad 2.

The rate at which mineral dissolves into the pore solution at a certain point, $v_{dis}$, is assumed to be proportional to the degree of undersaturation with respect to apatite. In our models two different measures for undersaturation have been used. In the first one of the undersaturation at a point is represented by the difference between the free energy of the undersaturated solution and that of the saturated solution, which is proportional to the difference between the negative logarithms of the ion product for apatite, $pI_A$, of the pore solution at that point and the solubility product of apatite $pK_A$ (Bennema and Gilmer, 1973). We then have:

$$v_{dis} = k_{dis} (pI_A - pK_A) \qquad 6.2.2$$

In the second one the undersaturation is represented by the difference of the concentration of a saturated solution and the pore solution (Linge and Nancollas, 1973) giving:

$$v_{dis} = k_{dis} (C_s - \bar{C}) \qquad 6.2.3$$

As the ratio of the total calcium and phosphate concentrations in solution will not be far from that in apatite we used formula 1.2.4 to calculate the two concentrations in 6.2.3 from the solubility product and the ion product respectively.

Finally we define the rate of mineral loss per unit of enamel surface as :

$$v_{dem} = \int_0^{\delta} v_{dis}\, dx \qquad 6.2.4$$

This parameter, which will be called the rate of demineralization, is an output parameter of the programs which can be determined by chemical methods in in vitro experiments.

ad 3.
In the mathematical models thirteen components are assumed to be present:

1. $K^+$ or $Na^+$
2. $Cl^-$
3. $H^+$
4. $Ca^{++}$
5. $H_2PO_4^-$
6. $H\ PO_4^=$
7. $H\ Z$
8. $Z^-$
9. $CaH_2PO_4^+$
10. $CaHPO_4^o$
11. $CaZ^+$
12. $OH^-$
13. $PO_4^{\equiv}$

The $OH^-$ and $PO_4^=$ ions are assumed to play a role in the ion product of apatite whereas their contribution as well as that of $H_3PO_4$ to the transport of matter is neglected. HZ stands for the acidic compound like acetic- or lactic acid.
Not all of the concentrations are independent. Most of them are related by equilibrium conditions. The corresponding chemical

reactions are assumed to reach equilibrium very fast as compared to all other processes of the caries process. If the general form of the equilibrium reaction is :

$$AB \overset{\leftarrow}{\rightarrow} A + B \qquad K_{AB}$$

then the relation between the activities of the components AB, A and B is:

$$\frac{a_A \quad a_B}{a_{AB}} = K_{AB} \qquad 6.2.5$$

The equilibria which have been incorporated in the models are:

| | | |
|---|---|---|
| 1. | $(H_3PO_4 \overset{\leftarrow}{\rightarrow} H^+ + H_2PO_4^-)$ | $K_1 = 7.59\ 10^{-3}$ |
| 2. | $H_2PO_4^- \overset{\leftarrow}{\rightarrow} H^+ + HPO_4^=$ | $K_2 = 6.17\ 10^{-8}$ |
| 3. | $HPO_4^= \overset{\leftarrow}{\rightarrow} H^+ + PO_4$ | $K_3 = 2.14\ 10^{-13}$ |
| 4. | $HZ \overset{\leftarrow}{\rightarrow} H^+ + Z^-$ | $K_4 = 1.78\ 10^{-5}$ |
| 5. | $CaH_2PO_4^+ \overset{\leftarrow}{\rightarrow} Ca^{++} + H_2PO_4^-$ | $K_5 = 1.95\ 10^{-1}$ |
| 6. | $CaHPO_4^o \overset{\leftarrow}{\rightarrow} Ca^{++} + HPO_4^=$ | $K_6 = 3.89\ 10^{-3}$ |
| 7. | $CaZ^+ \overset{\leftarrow}{\rightarrow} Ca^{++} + Z^-$ | $K_7 = 1.70\ 10^{-1}$ |
| 8. | $H_2O \quad H^+ + OH^-$ | $K_w = 10^{-14}$ |

The values of the equilibrium constants are taken from Moreno and Zahradnik (1974) ($K_4$ and $K_7$ for acetic acid). Of the thirteen concentrations only five are independent. An example of a set of concentrations which are independent is:

a. $K^+$ or $Na^+$
b. $Ca^{++}$
c. $H^+$
d. $H_2PO_4^-$
e. $Z^-$

The concentrations of all other calcium, phosphate and organic

acid containing compounds can be calculated from these concentrations. The concentration of $Cl^-$ can be calculated from the concentrations of the other twelve compounds, the fixed charge and the condition of electroneutrality.
In the Nernst-Planck flux equations, in the equilibrium equations and in the ion products the activity coefficients or their gradient appear.
As the models are not meant for quantitative purposes, Guntelbergs formula (Robinson and Stokes, 1959) is supposed to be accurate enough.

$$\log f_i = - Z_i^2 A \frac{\sqrt{I}}{1 + \sqrt{I}} \qquad 6.2.6$$

The three basic physicochemical processes, which are mathematically described by the equations 2.1.5, 6.2.1, 6.2.2 or 6.2.5, have been linked together into two different types of caries models. The first one is a static model with which a quasi steady state can be calculated. The second one is a dynamic simulation model with which the caries process can be simulated as a function of time.

There is, in principle, nothing that can be calculated with the dynamic model and not with the steady state model. The dynamic model needs, however, much more computer time than the steady state model. Caries is a dynamic process in which important system parameters like the mineral content change with time and thus the ultimate answers must be given by a dynamic caries simulation program, but the steady state model is very useful in calculating 'snap shots' of the dynamic process.

## *5.3 A Static Caries Model.*

The static model and the computer program FLUX5 (appendix E)

which is based on it are developed from the method for a general integration of the Nernst-Planck flux equation and the program FLUX which is described in section 2.3 and appendix C. The only essential difference is that in the caries model the various diffusing species and the membrane itself are involved in chemical reactions. This means that the flux of each ion or molecule needs not to be constant throughout the entire thickness of the membrane. The condition which must be satisfied in this case is that the concentrations of the various compounds do not change with time at any point $x_j$ within the membrane.

$$\frac{d\,\bar{C}_{ij}}{dt} = 0 \qquad 6.3.1$$

This condition is in fact a more general form of the condition used in FLUX. The rate at which the concentration of compound i changes with time is determined by two factors, (1) the diffusion and (2) the dissolution which are represented by 6.2.1 and 6.2.2 or 6.2.3 respectively, resulting in the combined equation:

$$\frac{d\,\bar{C}_{ij}}{dt} = -\frac{1}{\alpha_j} \cdot \frac{dJ_{ij}}{dx} + \nu_i\, v_{dis\ j} \qquad 6.3.2$$

This formula only applies to the total concentrations of all $H^+$ containing compounds (excluding water), all phosphate, all calcium and all organic acid compounds as well as to the concentration of the alkali and chloride. This is because a phosphate ion that leaves the mineral can, like a calcium or a hydroxyle ion, take several forms in the solution. In the formula $\nu_i$ means the number of ions of component i in one molecule of apatite. These are 10, 6 and -2 for calcium phosphate and $H^+$ containing compounds respectively (see section 1.2).

The most relevant input parameters of the model are:

1. the concentration of the various compounds in the bulk solutions;
2. the fixed charge of enamel at each point in the membrane;
3. the solubility product of the apatite at each point;
4. the dissolution rate constant $K_{dis}$ at each point;
5. the fractional pore volume at each point;
6. the diffusion coefficient of each compound at each point.

The most relevant output parameters are, at each point:

1. the fluxes of all components;
2. the concentration of all components;
3. the dissolution rate;
4. the ion products for apatite DCPD and OCP and the rate of demineralization (formula 6.2.4).

## *6.4 A Dynamic Caries Model.*

In the dynamic model the concentrations, the rate of demineralization and the total amount of apatite dissolved are calculated by integrating the various rates of change. The rate of change of concentrations at the various points inside the enamel is given by equation 6.3.2. As pointed out in section 6.2, only five concentrations are mathematically independent. Thus at each grid point $x_j$ five integrals for the concentrations have to be solved and one for the demineralization. The five independent concentrations we have chosen are :

a. $[Na^+]$ or $[K^+]$

b. $[Ca_{tot}] = [Ca^{++}] + [CaH_2PO_4^+] + [CaHPO_4^o] + [CaZ^+]$

c. $[P_{tot}] + [H_2PO_4^-] + [HPO_4^=] + [CaH_2PO_4^+] + [CaHPO_4^o]$

d. $[H_{tot}] = 2[H_2PO_4^-] + [HPO_4^=] + 2[H_2PO_4^+] + [CaHPO_4] + 2[CaH_2PO_4^+] +$

e. $[Z_{tot}] = [HZ] + [Z^-] + [CaZ^+]$

The parameter $\nu$ is for these five concentrations 0, 10, 6, -2 and 0 respectively. The calculation of the separate concentrations from the total concentration must be done by solving a nonlinear system of four equations. This is done readily by a Newton-Raphson iterative procedure (Stoer, 1972a), despite the fact that the so called condition of this set of equations is not too good (Stoer, 1972b). The integration of the system of differential equations is,of course, done numerically. The algorithm for each integration step consists of the following intermediate steps:

1. calculation of the thirteen separate concentrations from the total concentrations by Newton Raphseon;
2. calculation of $\frac{dC_i}{dx}$ by numerical differentiation (second degree interpolation);
3. calculation of the fluxes with 2.2.1;
4. calculation of $v_{dis}$ with 6.2.2 or 6.2.3;
5. calculation of $\frac{dC}{dt}$ for the five independent concentrations with 6.3.2.

The model is programmed using the Continuous Simulation Modeling Program, CSMPIII, of IBM (1975).

The most important input parameters are:

1. the concentrations of the various compounds in the bulk solutions;
2. the fixed charge of enamel at various points inside the enamel;
3. the solubility product of enamel at various points;
4. the dissolution rate constant;
5. the fractional pore volume at various points;
6. the diffusion coefficients of the various compounds.

The most important output parameters are as a function of time:

1. the concentration of the various compounds at several points in the enamel;
2. the dissolution rate at several points inside the enamel;
3. the total amount of enamel dissolved at various points inside the enamel;
4. the rate of demineralization (formula 6.2.4).

By the use of control parameters the simulation program can be made to choose between the two formulas for the dissolution rate (6.2.2 and 6.2.3).
Also by the use of control parameters the solubility product of the enamel apatite and the dissolution rate constant can be made functions of the concentrations of the various components. Indications for such a dependence are found in the literature (Driessens et al., 1977). The diffusion coefficients are made a function of the mineral content using a simplified form of the formula of Faxen (House, 1974) for the diffusion coefficient in porous media.

## *6.5 Results.*

In all mathematical simulations presented in this study the solubility product of the enamel apatite was supposed to be constant ($10^{-113}$) and the effective fractional water content was considered to be 10 percent by volume. Except for the simulation in which the influence of the selectivity on the rate of demineralization was investigated, the fixed charge was supposed to be zero and the ratios of the various diffusion coefficients were assumed equal to those in bulk water.

The concentrations which served as input for the first

Table 6.5.1.

Concentrations at the two boundaries in mmol $l^{-1}$ as well as the pH and the negative logarithm of the ion product for hydroxylapatite as used in most caries simulations. KCl was assumed to be present as an indifferent electrolyte with a concentration of up to 50 mmol $l^{-1}$.

| | enamel surface | dentino-enamel junction |
|---|---|---|
| Ca-tot. | 7.4 | 0.31 |
| $PO_4$-tot. | 4.6 | 0.19 |
| Ac-tot. | 50.0 | 0.01 |
| pH | 5.0 | 7.0 |
| $pI_A$ | 118 | 113 |

series of simulations with the steady state caries simulation program FLUX5 (appendix E) are listed in Table 6.5.1. A range of values for the dissolution rate constant and for the diffusion coefficients are used. The resulting rates of demineralization (formula 6.2.4) are listed in Table 6.5.2. In figure

Table 6.5.2.

Rate of demineralization (formula 6.2.4) in mol $l^{-1}$ $cm^{-2}$ $s^{-1}$ as a function of the dissolution rate constant, $k_{dis}$ in mol $l^{-1}$ $s^{-1}$, and the diffusion coefficients, $\bar{D}$ in $cm^2$ $s^{-1}$.

| $k_{dis}$ | $\bar{D}$ $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ |
|---|---|---|---|---|
| $10^{-10}$ | $8.07\ 10^{-14}$ | $8.06\ 10^{-14}$ | $7.98\ 10^{-14}$ | $7.29\ 10^{-14}$ |
| $10^{-9}$ | $8.06\ 10^{-13}$ | $7.98\ 10^{-13}$ | $7.29\ 10^{-13}$ | $4.13\ 10^{-13}$ |
| $10^{-8}$ | $7.98\ 10^{-12}$ | $7.29\ 10^{-12}$ | $4.13\ 10^{-12}$ | $1.08\ 10^{-12}$ |
| $10^{-7}$ | $7.29\ 10^{-11}$ | $4.13\ 10^{-11}$ | $1.08\ 10^{-11}$ | $2.83\ 10^{-12}$ |
| $10^{-6}$ | $4.13\ 10^{-10}$ | $1.08\ 10^{-10}$ | $2.83\ 10^{-11}$ | $8.93\ 10^{-12}$ |
| $10^{-5}$ | $1.08\ 10^{-9}$ | $2.83\ 10^{-10}$ | $8.93\ 10^{-11}$ | $3.37\ 10^{-11}$ |

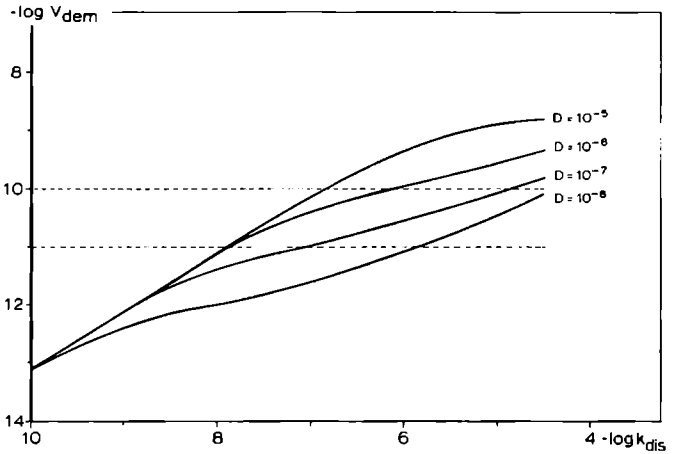


Figure 6.5.1.

The negative logarithm of the rate of demineralization in mol $cm^{-2}$ $s^{-1}$ ($-\log v_{dem}$) as a function of the negative logarithm of the dissolution rate constant in mol $l^{-1}$ $s^{-1}$ ($-\log k_{dis}$) for different diffusion coefficients in $cm^2$ $s^{-1}$ ($\bar{D}$). The range of values for the rate of demineralization which appeared to be experimentally relevant is indicated by the broken lines.

6.5.1 these rates are plotted for various values of the diffusion coefficients as a function of the dissolution rate constant. From the measurements of Holly and Gray (1968) and of Groeneveld and Arends (1975) it can be deduced that the rate of demineralization in artificial caries experiments will lie between 10 and 100 pmol $cm^{-2}$ $s^{-1}$ ($10^{-11}$ - $10^{-10}$ mol $cm^{-2}$ $s^{-1}$). This experimentally relevant range of demineralization rates is indicated by the broken lines in figure 6.5.1. This, together with the known ranges for the diffusion coefficients (Borggreven, Van Dijk and Driessens, 1977; Burke and Moreno, 1975), limits the range of values for the unknown dissolution rate constant

between $10^{-8}$ and $10^{-5}$ mol $l^{-1}$ $s^{-1}$. If the rate of dissolution (formula 6.2.2) is plotted against the x-coordinate (depth), the demineralization appears to be not of the subsurface type. If, however, a small gradient is introduced in the dissolution rate constant the demineralization becomes a subsurface one. As an example we have assumed this constant at the surface to be one fifth of the value in the bulk and to rise gradually to the bulk value. At the same time the ratio of the dissolution rate constant and the diffusion coefficient must be less than about $3.10^{0}$. In figure 6.5.2 the rates of dissolution are plotted as a function of the x-coordinate for three different values of this ratio. The rates have been normalized such that their maximum value is 1 to show the effect of the ratio on the site of maximum dissolution rate i.e. the depth of the lesion.

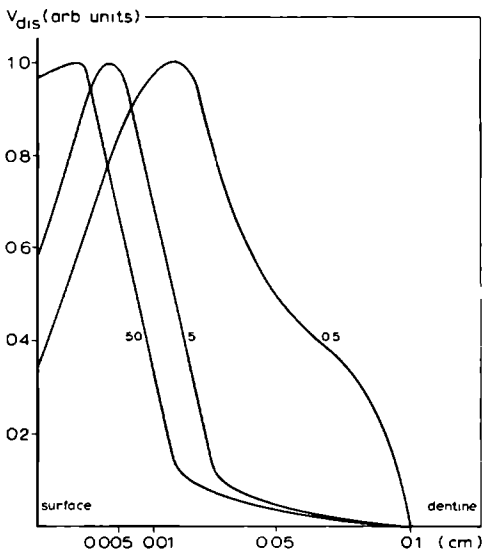


Figure 6.5.2.
The rate of dissolution ($v_{dis}$) as a function of the x-coordinate (depth in enamel) for three values of the ratio of the dissolution rate constant (in mol $l^{-1}$ $s^{-1}$) and the diffusion coefficient (in $cm^2$ $s^{-1}$). (The values for the bulk of the enamel are mentioned in the figure).

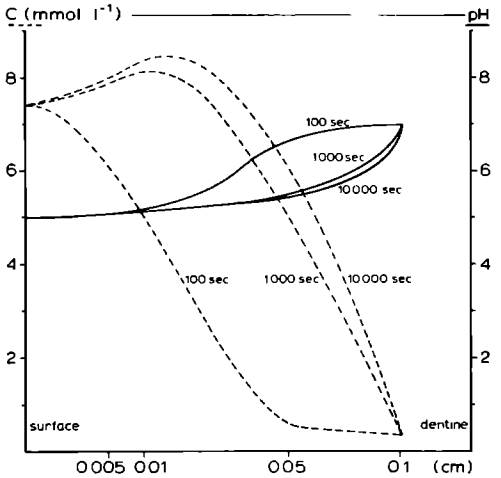


Figure 6.5.3.
The concentration of calcium in mmol $l^{-1}$ (dotted lines) and the pH (solid lines) as a function of the depth in enamel at 100, 1000 and 10.000 seconds after the start of the simulation. The values for the diffusion coefficients and of the dissolution rate constant were taken $3.10^{-7}$ $cm^2$ $s^{-1}$ and 1 to $5.10^{-7}$ mol $l^{-1}$ $s^{-1}$ respectively.

Figure 6.5.3 shows the profile of the total calcium concentration and that of the pH within the enamel at various instants. Figure 6.5.4 shows, for the same simulation, the profile of the dissolution rate. The simulation was carried out with the dynamic Caries Simulation Program CASIM (appendix F).

Still with the same input parameters the influence of the fixed charge of enamel and its ion selectivity (chapters 4 and 5) has been investigated. Four simulations were done:

1. fixed charge zero and ratios of diffusion coefficients as in bulk water;

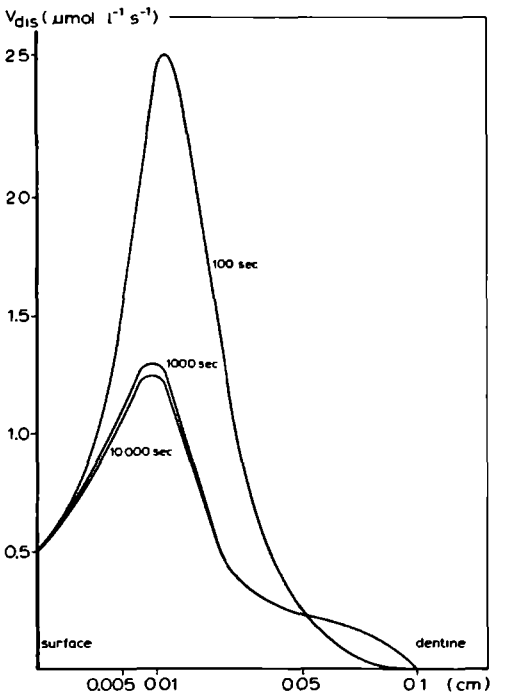


Figure 6.5.4.
The rate of dissolution in µmol $l^{-1}$ $s^{-1}$ as a function of the depth at 100, 1000 and 10.000 seconds after the start of the simulation. The values for the diffusion coefficients and of the dissolution rate constant were taken $3.10^{-7}$ $cm^2$ $s^{-1}$ and 1 to $5.10^{-7}$ mol $l^{-1}$ $s^{-1}$ respectively.

2. fixed charge + 20 meq $l^{-1}$ and ratios of diffusion coefficients as in bulk water;
3. fixed charge - 20 meq $l^{-1}$ and ratios of diffusion coefficients as in bulk water;

4. fixed charge - 20 meq $l^{-1}$ and all diffusion coefficients of cations multiplied by two and those of anions divided by two.

Table 6.5.3.
Simulation of caries with ion selective enamel. $k_{dis}/\bar{D}$ is the ratio of the dissolution rate constant in mol $l^{-1}$ and the diffusion coefficient in $cm^2$ $s^{-1}$. $\bar{D}_+/\bar{D}_-$ is the ratio of diffusion coefficients of cations and anions relative to that in bulk water. $\omega\bar{X}$ is the fixed charge in meq $l^{-1}$, $d_l$ the lesion depth in micro meter and $v_{dem}$ the rate of demineralization normalised to 1 for the neutral case.

| $k_{dis}/\bar{D}$ | | 5 $10^{-2}$ | | 5 $10^{-1}$ | | 5 $10^{0}$ | |
|---|---|---|---|---|---|---|---|
| $\bar{D}_+/\bar{D}_-$ | $\omega\bar{X}$ | $d_l$ | $v_{dem}$ | $d_l$ | $v_{dem}$ | $d_l$ | $v_{dem}$ |
| 1 | 0 | 800 | 1.0 | 130 | 1.0 | 10 | 1.0 |
| 1 | + 20 | 800 | 1.0 | 130 | 1.0 | 10 | 1.0 |
| 1 | - 20 | 800 | 1.0 | 130 | 1.0 | 10 | 1.0 |
| 4 | - 20 | 800 | 0.9 | 120 | 0.8 | 0 | 0.8 |

The results are listed in Table 6.5.3 in which the demineralization rate is normalised to one for the neutral case (the first one). The calculations were done with three different values for the ratio of the dissolution rate constant and the diffusion coefficients: 0.5, 5 and 50. The results did not differ more than a few percent. In the table the lesion depths are also listed.

In the simulation experiments described below the outside solution is assumed to contain no calcium and phosphate. They

were set up to simulate the in vitro caries experiments of Holly and Gray (1968) and of Groeneveld and Arends (1975).

Holly and Gray (1968) did experiments in which they used either a 0.1 mol $l^{-1}$ acetate buffer or a 0.1 mol $l^{-1}$ lactate buffer, both of pH = 4.5 and containing 6 percent hydroxyaethylcellulose (HEC). As the HEC contains impurities that protect the surface layer for dissolution (Francis, Briner and Gray, 1973) a gradient in the dissolution rate constant was assumed.

Table 6.5.4.
Simulation of the experiments of Holly and Gray (1968) with acetate and lactate. $k_{dis}$ is the dissolution rate constant in mol $l^{-1}$ $s^{-1}$, $\bar{D}$ is the diffusion coefficient in $cm^2$ $s^{-1}$ and $k_{dis}/\bar{D}$ is their ratio. The lesion depth, $d_l$, is in micrometers and the rate of demineralization is in pmol $cm^{-2}$ $s^{-1}$.

| $k_{dis}/\bar{D}$ | 5 $10^{-1}$ | | 5 $10^{-1}$ | | 5 $10^{0}$ | | | |
|---|---|---|---|---|---|---|---|---|
| $k_{dis}$ | 2.4 $10^{-8}$ | | 6.6 $10^{-8}$ | | 2.7 $10^{-7}$ | | Exp | |
| $\bar{D}$ | 4.7 $10^{-7}$ | | 1.3 $10^{-7}$ | | 5.3 $10^{-8}$ | | | |
| | $d_l$ | $v_{dem}$ | $d_l$ | $v_{dem}$ | $d_l$ | $v_{dem}$ | $d_l$ | $v_{dem}$ |
| acetate | 150 | 36 | 75 | 36 | 0 | 36 | 100 | 36 |
| lactate | 150 | 34 | 75 | 26 | 0 | 28 | 100 | 37 |

In Table 6.5.4 the results of simulations with three different values for the ratio of the dissolution rate constant and the diffusion coefficients are listed. The diffusion coefficients were chosen such that the rates for the acetate experiments correspond to the experimental values. The values for the diffusion coefficients appear to be about $10^{-7}$ $cm^2$ $s^{-1}$ and that for the dissolution rate constant $10^{-7}$ mol $l^{-1}$ $s^{-1}$ to fit to the experimental data.

Groeneveld and Arends (1975) did a series of artificial caries experiments with 0.1 mol $l^{-1}$ lactate buffers containing HEC. They adjusted the pH of the buffers to 4, 4.5 or 5 and measured the development of the subsurface lesions by quantitative microradiography. In Table 6.5.5 the results of the similations for three different ratios of the dissolution rate constant and the diffusion coefficients are listed. The values of the diffusion coefficients were chosen such that the rate of demineralization in the simulation is the same as the experimental value for the experiments at pH = 5.0. The values for $\bar{D}$ and $k_{dis}$ appear to be about $5.10^{-8}$ $cm^2$ $s^{-1}$ and $3.10^{-8}$ mol $l^{-1}$ $s^{-1}$ respectively.

Table 6.5.5.

Simulation of experiments of Groeneveld and Arends (1975). $k_{dis}$ is the dissolution rate constant in mol $l^{-1}$ $s^{-1}$, $\bar{D}$ the diffusion coefficient, in $cm^2$ $s^{-1}$ and $k_{dis}/\bar{D}$ is their ratio. The lesion depth, $d_l$, in micrometers and the rate of demineralization, $v_{dem}$, in pmol $cm^{-2}$ $s^{-1}$ are listed for both the simulations and the actual experiment.

| $k_{dis}/\bar{D}$ | 5 | $10^{-2}$ | 5 | $10^{-1}$ | 5 | $10^{0}$ | Exp | |
|---|---|---|---|---|---|---|---|---|
| $k_{dis}$ | 6.0 | $10^{-9}$ | 2.3 | $10^{-8}$ | 8.5 | $10^{-8}$ | | |
| $\bar{D}$ | 1.2 | $10^{-7}$ | 4.5 | $10^{-8}$ | 1.7 | $10^{-8}$ | | |
| | $d_l$ | $v_{dem}$ | $d_l$ | $v_{dem}$ | $d_l$ | $v_{dem}$ | $d_l$ | $v_{dem}$ |
| pH = 4 | 150 | 11.4 | 140 | 14.5 | 30 | 14.2 | 144 | 21.2 |
| pH = 4.5 | 150 | 8.4 | 80 | 9.0 | 5 | 8.9 | 84 | 8.2 |
| pH = 5 | 120 | 5.1 | 40 | 5.1 | 0 | 5.1 | 75 | 5.1 |

*6.6 Discussion.*

Holly and Gray (1968) used their model to explain the rate of demineralization of their in vitro caries experiments. The authors succeeded in finding such values for the parameters of their model that the rates predicted by the model correspond to the experimentally found values. Their model can, however, not explain the development of a subsurface lesion. The authors do not explain why they think that the rate of diffusion of the acid into the lesion is the rate determining factor, and not the rate of diffusion of the other components which are important in caries as well.

Although the model of Zimmerman (1966) is more sophisticated than that of Holly and Gray (1968) he did not succeed in simulating the development of a subsurface lesion. The main reson for this is that the rate of dissolution was assumed to be very fast as compared to the diffusion processes. This corresponds with a large ratio of the dissolution rate constant and the diffusion coefficients. If we use a large ratio (larger than $10^3$ for example) unrealistic large gradients in the dissolution rate constant or in the solubility product of apatite must be introduced to save the superficial layer.

From the presented data it can be deduced that, if it is assumed, that the dissolution rate is not very fast as compared to longitudinal diffusion, it is possible to simulate the development of a subsurface lesion by introducing a moderate gradient in (1) the dissolution rate constant. A similar effect has a gradient in (2) the solubility product of the enamel apatite (Driessens, 1973) and (3) a gradient in the porosity of enamel (Bakhos, Brudevold and Aasenden, 1976) or a correctly chosen combination of these.

The ratio of the rate constant of the dissolution process and of the diffusion coefficients appears to be an important parameter of the model. Changing this parameter in such a way that the rate of demineralization does not change, which can be done using figure 6.5.1, will for the same system shift the point at which the dissolution rate is maximal. In other words the ratio is important in determining the depth of the lesion, the larger it is, the thinner the surface layer (figure 6.5.2). The development of the profiles for the concentrations inside the enamel is roughly as can be expected. The pH as a function of the x-coordinate needs perhaps some explanation. The pH remains quite constant as a function of x and rises only in the very last part of the penetrated enamel. This is due to the fact that the diffusion coefficients of the dissociated acid and the undissociated acid do not differ considerably and thus the buffer as such is diffusing into the enamel. As a consequence the pH only starts to rise where the concentration of the buffer becomes too low to have a larger buffer capacity than the phosphate originally present in the pore liquid.

To obtain a subsurface lesion it is not only necessary that the region in which the rate of dissolution is largest lies within the enamel in the quasi steady state, but also this steady state must be established so fast that no considerable quantities of the surface enamel can be dissolved in this period. Figure 6.5.4 shows that the quasi steady state is indeed reached very soon, well within the periods of metabolic activity of the plaque (20 minutes and more, Graf 1969).

The comparison of the results of the computer simulations with the experimental results of Holly and Gray (1968) and of Groeneveld and Arends (1975) show an encouraging agreement. They

all indicate the same order of magnitude for the dissolution rate constant and for the diffusion coefficients. We think that the best value for the rate constant is between $10^{-8}$ and $10^{-7}$ mol $l^{-1}$ $s^{-1}$ and for the diffusion coefficients between $10^{-8}$ and $10^{-7}$ $cm^2$ $s^{-1}$. This last observation is in good agreement with experimental data of Borggreven, Van Dijk and Driessens (1977) especially if one considers the rise in the diffusion coefficient after some demineralization of the enamel.

From the theories of Bennema and Gilmer (1973) the activation energy and the half life of the dissolution process can be estimated to be between 96 and 113 kJ $mol^{-1}$ and $10^2$ and $10^3$ seconds respectively. These values correspond quite well with data of Kibby and Hall (1972) and of Pak and Bartter (1967) respectively.

The quantitative aspects of the results need some discussion. The figures in the tables suggest an accuracy which is of course not meant as physically real. One must look at these figures as indications of their order of magnitude.

Some input parameters were used without comment. The most important of which is the solubility product of enamel. This is assumed to be constant and $10^{-113}$ neither of which will be true in general (Patel and Brown, 1975).
Taking other values between $10^{-105}$ and $10^{-120}$, however, will not change the output variables with more than a factor ten.

Another point which is introduced without comment is the use of formula 6.2.2 for the rate of dissolution and not one of the type 6.2.3 which is more commonly met in the apatite and enamel literature. The reason for this is that the mathematical stability of the program is better with 6.2.2 and the results, in terms of orders of magnitude and trends, are not very different with both formulas. But if more quantitative models are to

be made these factors deserve further attention.

The results of the simulations in which the effect of the ionselectivity is studied (Table 6.5.3) show that affecting this selectivity will not reduce the rate of the caries process in a clinically significant way. This leads also to the conclusion that the reduction of caries by fluoride or possibly by the phosphates mentioned in the previous chapter will not be caused by their effect on the ionselectivity of enamel. Brown (1974) argues that the fixed charge of enamel could affect the rate of the caries process by changing the ratios of the concentrations of the proton donors and proton acceptors. As these factors are taken into account in our models it seems that they are not very important as determining factor for the rate of the caries process.

In conclusion the mathematical model presented in this study has proved to be a reasonable description of in vitro caries experiments. The simulations indicate that it is improbable that subsurface lesion will occur unless one or a combination of the following conditions is satisfied:

1. there is a gradient in the solubility product of the enamel apatite;
2. there is a gradient in the dissolution rate constant;
3. there is a gradient in the water content of the enamel.

Both the rate of the diffusion process and the rate of the dissolution process determine the rate of demineralization. It is not correct to assume one of these processes very fast as compared to the other (Holly and Gray, 1968; and Zimmerman, 1966).

If physically reasonable values of the rate constants of these processes are assumed, the above mentioned gradients need only to be moderate for obtaining subsurface demineralization. It is improbable that the rate of the caries process can be

reduced in a significant way by affecting the fixed charge, or more generally the ionselectivity of the enamel, provided this ionselectivity only affects the longitudinal diffusion.

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# 7 IN VITRO CARIES EXPERIMENTS.

## *7.1 Introduction.*

Several investigators have carried out experiments in which they tried to simulate the natural caries process in an in vitro system. These experiments have been done for a number of reasons: (1) for getting a better understanding of the mechanism of the caries process, (2) for the testing of potential anticariogenic compounds and (3) for studying remineralization.

The first investigator who succeeded in obtaining artificially a caries like subsurface lesion was von Bartheld (1958, 1961). As already mentioned in section 1.3 he supposed an acidified charged gel to be necessary to obtain a subsurface demineralization. However, other investigators (among which Groeneveld and Arends, 1975) were able to obtain caries like lesions using acidified uncharged gels.

No gels were used by Moreno and Zahradnik (1974) and by Joost Larsen (1974). In the experiments of Moreno and Zahradnik the formation of a second solid phase, DCPD, was suggested to be responsible for the occurrence of a subsurface lesion. In the experiments of Joost Larsen the incorporation of fluoride in the apatite lattice, which causes a gradient in the solubility product of the apatite, can be responsible for the relative stability of the surface layer.

Francis, Briner and Gray (1973) did experiments in which they added polyphosphates, diphosphonates or phytate to the acidic buffer. These compounds obviously protect the surface layer of the enamel. They found subsurface demineralization when one of the compounds was present and otherwise they found etching of the enamel.

It seems that none of these studies could reveal the minimum set of conditions necessary to obtain a subsurface lesion in dental enamel.

The results of the computer simulations described in the previous chapter did suggest that the, from a mechanistic point of view, most simple way of getting subsurface caries like lesions is to use an acidic buffer which is more or less saturated with respect to apatite. No additional requirements seem to ne necessary. To investigate the validity of this hypothesis, experiments with acetate buffers which were more or less saturated with respect to synthetic hydroxylapatite were carried out. They are described in this chapter.

## *7.2 Materials and Methods.*

The buffers used in the in vitro caries experiments were made by adding potassium hydroxyde to a 50 mmol $l^{-1}$ acetic acid solution until the pH of 5.0 was attained. To part of the buffer synthetic hydroxylapatite was added. The mixture was stirred during fourteen days at room temperature. This saturated buffer was mixed with the original to obtain acetate buffers which were 80, 60 and 40 percent saturated with hydroxylapatite. Before mixing, the original buffer was carefully adjusted to the same pH as the saturated one.

The amount of phosphate in the buffers has been determined by isotachophoresis (Everaerts, Beckers and Verheggen, 1976). The results of these measurements are summarized in table 7.2.1. The third and fourth column of the table contain the negative logarithms of the ion products for apatite and DCPD respectively. These values are calculated assuming that the hydroxylapatite was dissolved congruently.

Table 7.2.1.
Phosphate concentration of the acetate buffers and the negative logarithm of the ion product for hydroxylapatite and dicalcium phosphate dihydrate.

| relative "saturation" | concentration in mmol $l^{-1}$ | $pI_A$ | $pI_D$ |
|---|---|---|---|
| 100% | 2.1 | 122 | 7.8 |
| 80% | 1.7 | 124 | 8.0 |
| 60% | 1.3 | 126 | 8.2 |
| 40% | 0.8 | 129 | 8.6 |

The apatite has been synthesized by Schaeken et al. (1975) by heating a mixture of $CaHPO_4$ and $CaCO_3$ at 1000°C. It was ground and reheated until X-ray diffractograms did not show the presence of second phases. The solubility product of the apatite was $1.5\ 10^{-112}$ (Verbeeck and Thun, 1977). From the values for the ion product for apatite (table 7.2.1) and this solubility product it can be seen that the 100 percent solution was not in equilibrium and thus the term saturated has only a relative meaning. All chemicals used were analytical grade, the water was demineralized and distilled twice.

The teeth used in the experiments were sound human molars which, on visual inspection, did not show signs of caries or significant cracks. The roots and pulp were removed and the surface of the crown was carefully cleaned by pumice and a tooth brush. After tohrough rinsing with distilled water the entire element was sealed with dental sticky wax except for two windows of 3 x 3 mm. During all stages of the preparation, except the sealing, the elements were kept wet to prevent dessiccation.

Each element was placed in a bottle with 25 ml of the acidic buffer. The bottles were agitated continuously on a shaker during either four or ten days. After the experiment was stopped the elements were sectioned perpendicular to the tooth surface in 100 µm thick slices. The sections were microradiographed using an X-ray source with tungsten anode, operated at 20 kV and 18 mA.

### *7.3 Results.*

Plates 7.3.1 and 7.3.2 show the results of the action of the buffers (section 7.2) on human molars. Each photograph represents an area of about 2 x 3 mm. The photographs were taken from the microradiographs of the sections of teeth exposed, from top left to bottom right, to the 100 percent 80, 60 and 40 percent buffer respectively.

The photographs clearly show that with decreasing degree of saturation of the outside solution the lesion depth increases and the thickness of the surface layer decreases. Such a layer was completely absent when the 40 percent solution was used. After ten days the lesion depth is greater than after three days. The thickness of the superficial layer, however, seemed to have increased.

### *7.4 Discussion.*

In the opinion of von Bartheld (1961) a Donnan distribution between the outside solution (the pellicle-plaque-saliva system) and the enamel is responsible for the fact that the demineralization of enamel is more severe at some distance from the surface than at the surface. The system used in the experiments of von Bartheld consisted of a gel, e.g. gelatine, which was

Plate 7.3.1.

Microradiographs taken after four days. The solutions used were 100 percent, 80, 60 and 40 percent saturated with respect to hydroxylapatite (top left to bottom right). Each photograph covers an area of about 2 x 3 mm.

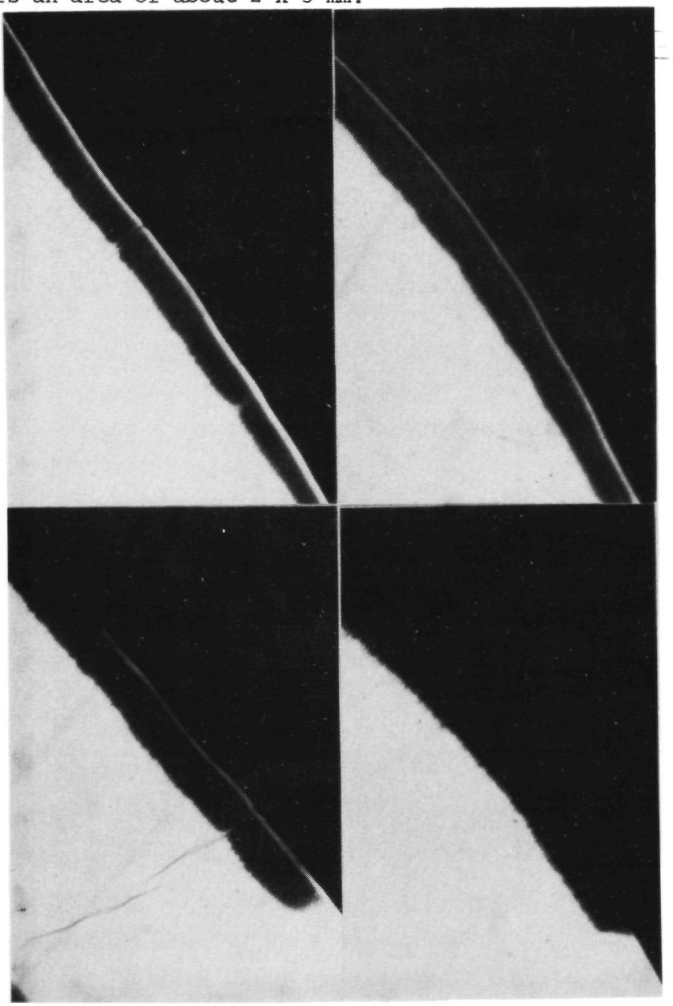
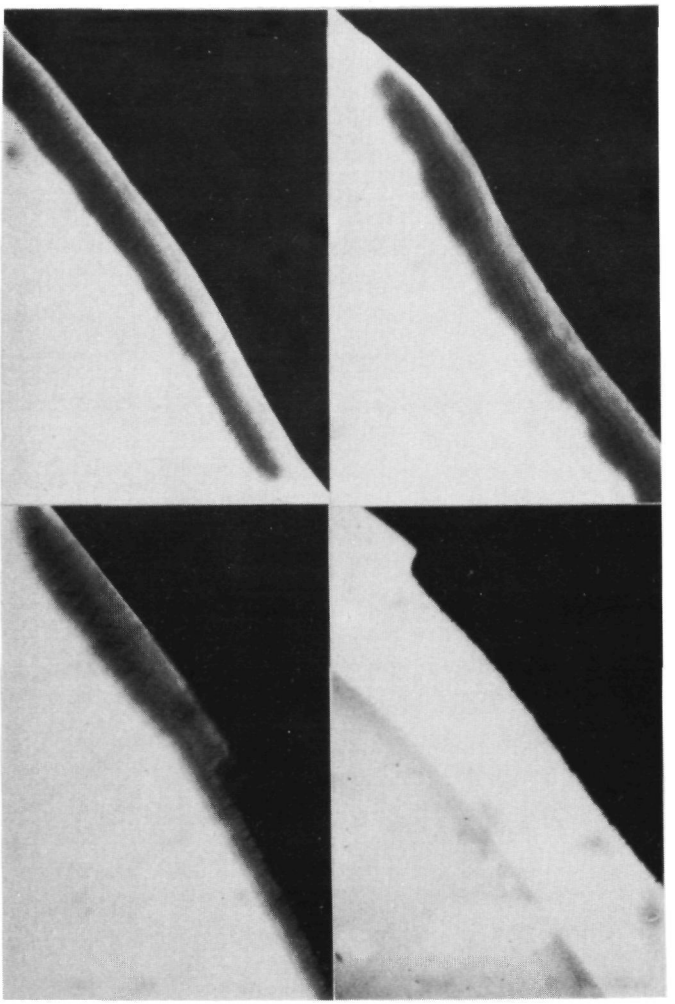


Plate 7.3.2.

Microradiographs taken after ten days. The solutions used were 100 percent, 80, 60 and 40 percent saturated with respect to hydroxylapatite (top left to bottom right). Each photograph covers an area of about 2 x 3 mm.



acidified with lactic acid to below its isoelectric point. Then such macromolecules bear a positive charge and as they cannot enter the enamel a Donnan equilibrium is established at the interface of gel and enamel (section 2.1). As a consequence the solution in the pores of enamel will contain more undissociated lactic acid relative to lactate than the outside gel. This is suggested to cause the subsurface character of caries.

Von Bartneld does not argue why the effect of the Donnan distribution should not start right at the surface but at a depth of ten or more micrometers. Moreover, the isoelectric point of the gelatine which was used in these experiments was 4.7, which means that if the gelatine is positively charged the pH was below 4.7. Moreno and Zahradnik (1974) showed that at these pH values dicalcium phosphate dihydrate (DCPD) is formed on the apatite crystals of enamel. This on its own could be responsible for the occurrence of subsurface demineralization in the experiments of von Bartheld.

Groeneveld and Arends (1975) did a number of in vitro caries experiments in which they used hydroxy-ethyl-cellulose (HEC) in stead of gelatine. This macromolecule can be supposed to be uncharged in the pH range of the experiments. Groeneveld and Arends obtained artificial caries like lesions in enamel using 0.1 mol $l^{-1}$ lactate buffers which contained six percent HEC. The pH of the gels used was adjusted to 4, 4.5 or 5. These experiments seem to disprove both the necessity of a charge on the gel molecules and that of the formation of DCPD as put forward by Moreno and Zahradnik (1974).

Francis, Briner and Gray (1974), however, have dialysed the HEC. When they used a buffer with the dialysed gel they found etching of the surface instead of a subsurface demineralization. When they used the dialysed gel with the dialysate

added again they found subsurface demineralization. Their conclusion was that impurities of the HEC protect the surface layer of the enamel. It is far from improbable that the gelatine used by von Bartheld also contained such impurities.

Francis, Briner and Gray (1974) also carried out experiments with lactate buffers of pH = 4.5 to which polyphosphates, phytic acid or a diphosphonate were added. Addition of about 0.1 mmol $l^{-1}$ of one of these compounds caused a subsurface lesion, without these compounds etching occurred. If impurities in HEC are of these kinds of compounds their concentration needs to be 0.1 percent only, which is quite realistic. This might, by the way, also be valid for the gels used by von Bartheld (1961).

The experiments of Francis et al. with the dialysed HEC have also greatly reduced the value of the in vitro caries experiments of Langdon, Dykes and Fearnhead (1976). These authors obtained subsurface lesions in hydroxylapatite pellets using HEC containing buffers. Otherwise their experiments would have disproved the theories in which some special property of enamel like a gradient in the solubility product (Driessens, 1973) is the cause of the relative stability of the surface layer.

The experiments which were described in the previous section of this chapter do not support the hypothesis of Moreno and Zahradnik (1974), that the formation of DCPD is necessary to get a subsurface demineralization instead of an etching of the enamel. The pH of our buffers was 5.0 which is higher than the pH = 4.7 below which Moreno and Zahradnik found DCPD to be more stable than enamel apatite. The experiments of Arends and Davidson (1975) also make the conclusion of Moreno and Zahradnik about the crucial role of DCPD questionable. They determined the $HPO_4^{2-}$ content of the mineral in both the superficial layer and in the body of the artificial lesion formed at pH = 4.5. They

found that it was higher in the lesion than in the surface layer covering it. This means that the DCPD, if formed, does not stabilize the surface layer more than it does the rest of the enamel.

It is very improbable that the relative stability of the superficial layer in our experiments is due to some impurity in the buffer. Acetic acid and potassium hydroxyde were analytical grade. The X-ray diffractograms made of the hydroxylapatite used, did not show impurities. This means that they are not present in quantities of more than one percent. As the apatite is synthesized at 1000$^{o}$C, pyrophosphate does not occur (Winand, 1965). The presence of higher polyphosphates is even less probable. Even if these compounds were present for 1 percent in the apatite, it would result in a concentration in the buffer of much less than 0.1 mmol $l^{-1}$ which is proved to be insufficient to protect the superficial layer by Francis, Brinex and Gray (1974).

Although the experimental conditions of our experiments do differ much from those of e.g. Groeneveld and Arends (1975), Francis, Briner and Gray (1974) and Holly and Gray (1968), the appearance of the lesions, both as a function of time and of distance from the surface, is analogous. The depth of the lesion increases with the time of exposure of the element to the buffer. The rate of demineralization in the centre of the lesion can be estimated to be between 1 and 10 percent of the mineral per day. This is also in agreement with the results of the computer simulations as discussed in the previous chapter (section 6.5).

In conclusion the results presented in this chapter as well as the results obtained with the simulation models (chapter 6) about subsurface demineralization indicate that:

1. there is no evidence that the formation of a second solid phase is necessary to explain the relative stability of the surface layer;
2. a gel, either charged or not, is not necessary to obtain a caries like subsurface demineralization.

From the mathematical model (chapter 6) it could be concluded, that the surface layer can be saved by factors like a gradient in the solubility product, in the dissolution rate constant and in the porosity of the enamel. The various experiments discussed in this chapter support the idea that, depending on the type of experiment, one or a combination of these factors was responsible for the subsurface character of the demineralization. The use of an acidic buffer solution which either is relatively saturated with enamel mineral or which contains a chemical agent protecting the mineral from dissolution will favour subsurface demineralization.

Finding the main physicochemical factors that determine the caries process and especially the occurrence of the subsurface lesion needs further and thorough investigation, whereby the use of mathematical simulations can be of great help.

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# 8 SAMENVATTING.

Deze studie behandelt de voor het cariësproces relevante fysisch chemische verschijnselen in tandglazuur. Speciale aandacht krijgt de rol van het ionentransport in glazuur.

In hoofdstuk 1 worden enige eigenschappen van glazuur besproken. Tandglazuur bestaat uit kleine kristalletjes van een of meerdere calciumfosfaten, ingebed in een organische matrix. Het calciumfosfaat dat het grootste deel van het glazuur vormt, heeft een samenstelling en een structuur verwant aan die van hydroxylapatiet. De matrix bestaat uit lipiden, eiwitten en water. In het apatietrooster kunnen substituties en vacatures voorkomen die het oplosbaarheidsproduct van het mineraal variabel maken. Bij het oplossen van glazuur zal complexvorming optreden van fosfaat- met calciumionen en protondonors wat de oplosbaarheid van glazuur een ingewikkelde functie maakt van de vloeistofsamenstelling. Hierbij is de pH van de oplossing een zeer belangrijke factor.

Tandbederf (cariës) is een ziekte waarbij mineraal uit het tandglazuur verloren gaat. Wil een tand aangetast worden door cariës dan moet aan een aantal voorwaarden gelijktijdig worden voldaan (König, 1971). Deze zijn aanschouwelijk gemaakt in fig. 1.3.1. Karakteristiek voor cariës is dat de demineralisatie, die veroorzaakt wordt door zuren geproduceerd door de microflora op het tandoppervlak, het ernstigst is op enige diepte onder het tandoppervlak. Dit veroorzaakt de kenmerkende onderhuidse lesie (plaat 1.3.1) die klinisch wordt gekenmerkt door een witte vlek ("white spot") op de tand. De gevoeligheid van tandglazuur voor cariës kan, in principe, worden verminderd door in te grijpen in een van de fysisch-chemische processen, die tijdens cariës

plaatsvinden in glazuur. Deze processen zijn:

(1) diffusie van ionen en moleculen door de poriën van glazuur;

(2) oplossen van mineraal en;

(3) complexatie van verschillende ionen in de oplossing in de poriën.

Het doel van deze studie was de invloed van het diffusieproces op de snelheid van het cariësproces te onderzoeken. Speciale aandacht kreeg de ionselectiviteit van glazuur en de wijze waarop deze kan worden beïnvloed.

In hoofdstuk 2 wordt de fundamentele fysische chemie van de transportprocessen in geladen membranen behandeld. Een reeks formules wordt afgeleid, waarmee uit de membraan eigenschappen en die van de bulkoplossingen, de membraan potentiaal berekend kan worden. Een methode wordt gegeven waarmee uit metingen van de electromotorische kracht (emf) de transporteigenschappen van een geladen membraan, bijvoorbeeld glazuur, berekend kunnen worden. Deze formules zijn geldig voor een systeem waarin slechts een enkelvoudig electroliet aanwezig is. Correcties voor activiteitscoëfficiënten worden gemaakt. Voor het berekenen van de transportverschijnselen in geladen membranen in ingewikkelder systemen werd eveneens een methode ontwikkeld.

In hoofdstuk 3 wordt het instrumentarium, de bereiding van oplossingen, de preparatie van glazuurplakjes en de meetmethode van de emf van een concentratiecel met een glazuur membraan behandeld. De stukjes glazuur die als membraan in de concentratiecellen dienden, waren 200 µm dikke plakjes, gezaagd uit juist niet doorgebroken rundersnijtanden. De electrodes die voor de emf-metingen werden gebruikt, waren thermoelectrolytische zilver-zilverchloride electrodes.

In hoofdstuk 4 worden de resultaten gepresenteerd van emf metingen aan runderglazuur dat niet behandeld was met een speci-

ale chemische stof. De elctrolyten die het meest gebruikt werden, zijn rubidiumchloride en magnesiumchloride. Het blijkt dat de met de in hoofdstuk 2 afgeleide formules berekende potentialen in goede overeenstemming zijn met de gemeten emf's. De resultaten duiden erop dat het runderglazuur een negatieve vaste lading heeft in de RbCl oplossingen en minder negatief of zelfs positief geladen wordt in de $MgCl_2$ oplossingen. Dit is in overeenstemming met de waarnemingen van Waters (Van Dijk et al., 1977) met KCl, NaCl en $CaCl_2$ oplossingen.

In hoofdstuk 5 worden de resultaten gepresenteerd van emf metingen aan runderglazuur voor en na een behandeling met een chemisch agens. De agentia die gebruikt werden, zijn: orthofosfaat, pyrofosfaat, tri-metafosfaat, hexa-metafosfaat, phytaat, 1,1 ethylhydroxydifosfonaat (EHDP), monofluorofosfaat (MFP) en fluoride. Alle behandelingen zijn gedaan met waterige oplossingen bij pH = 7. Behalve orthofosfaat konden alle agentia de ionselectiviteit min of meer blijvend verhogen. De toename varieerde van 110 tot meer dan 500 percent van de oorspronkelijke waarde. Het minst succesvol waren pyrofosfaat, tri-metafosfaat en fluoride. Zeer succesvol was phytaat. MFP deed de ionselectiviteit veel meer stijgen en met een grotere persistentie dan zowel orthofosfaat als fluoride.

In hoofdstuk 6 wordt een wiskundig model voor het cariësproces behandeld. Het model omvat:

(1) diffusie van ionen en moleculen;
(2) oplossen en neerslaan van mineraal en;
(3) complexatie van de verschillende ionen die in het systeem aanwezig zijn.

Met computerprogramma's die op het wiskundig model gebaseerd zijn, is het mogelijk de ontwikkeling van een onderhuidse lesie

te simuleren. De belangrijkste condities voor het onderhuids doen worden van een lesie blijken te zijn:

(1) de snelheden van de diffusieprocessen en van het oplosproces moeten van vergelijkbare omvang zijn en;

(2) de bulkoplossing moet bij de gebruikte pH een buffercapaciteit hebben groter dan die van het fosfaat dat in de oplossing aanwezig is en;

(3) er moet een gradient zijn in of het oplosbaarheidsproduct of de oplossnelheidsconstante of de porositeit van het glazuur.

De computersimulaties zijn in kwantitatieve overeenstemming met in-vitro cariës experimenten vermeld in de literatuur. Uit deze experimenten en de simulaties kunnen waarden afgeleid worden voor de snelheidsconstantes van de fysisch-chemische processen die optreden gedurende cariës.

In hoofdstuk 7 worden een aantal in-vitro cariësexperimenten beschreven. Het doel van deze experimenten was om de minimum reeks van condities te vinden die nodig is om kunstmatig in glazuur een cariësachtige ontkalking te verkrijgen. De experimenten zijn gedaan met 50 mmol $l^{-1}$ acetaat buffers met pH = 5.0, die min of meer verzadigd waren met synthetisch hydroxylapatiet. Gedurende de experimenten werd de oplossing geagiteerd.

We concludeerden uit deze experimenten dat het gebruik van een (geladen) gel of de toevoeging van speciale agentia aan de buffer niet nodig is om een onderhuidse ontkalking te verkrijgen. Deze experimenten ondersteunen evenmin de theorieën die de vorming van een tweede vaste fase op het kristaloppervlak noodzakelijk vooronderstellen. Onderhuidse ontkalking trad op indien de oplossingen meer dan ongeveer 1 mmol $l^{-1}$ calcium

en fosfaat bevatten.

Het vinden van de belangrijkste fysisch-chemische factoren die het cariësproces en in het bijzonder het optreden van de onderhuidse ontkalking bepalen, benodigt een verder en grondig onderzoek waarbij het gebruik van wiskundige simulaties van veel nut zal kunnen zijn.

## SUMMARY.

This investigation deals with physicochemical phenomena in dental enamel, relevant for the caries process. Special attention is paid to the role of the transport of ions in dental enamel.

In chapter 1 some properties of dental enamel are discussed. Dental enamel consists of tiny crystals of one or more calciumphosphates embedded in an organic matrix. The calcium phosphate that forms the major part of the enamel, has a composition and structure related to that of hydroxylapatite. The matrix consists of lipids, proteins and water. Substitutions can take place and vacancies can occur in the apatite lattice, which make the solubility product of the mineral variable. On dissolution of enamel, complexation reactions of phosphate with calcium ions and protondonors make the solubility of enamel a complex function of the composition of the solution. In this respect the pH of the solution is a very important factor.

Caries is a disease in which mineral is lost from the enamel. For a tooth to be affected by caries a number of conditions must be fullfilled simultaneously (Kõnig, 1971). They are visualised in fig. 1.3.1. Characteristic for caries is that the demineralization, caused by the acids produced by the microflora on the tooth surface, is strongest at some distance under the surface. This causes the characteristic subsurface lesion, plate 1.3.1 which is clinically characterised by a white spot on the tooth surface.

The caries sensitivity of dental enamel can, in principle, be reduced by affecting one of the physicochemical processes that occur in enamel during caries. These processes are: (1) diffusion of ions and molecules through the pores in enamel (2) dis-

solution of mineral and (3) complexation of the various ions in the pore solution. The aim of this study was to investigate the influence of the diffusion process on the rate of the caries process.
Special attention was paid to the ionselectivity of dental enamel and how it can be influenced.

In chapter 2 the basic physicochemistry of the transport processes in charged membranes is discussed. A set of formulas is derived for the calculation of the membrane potential from the membrane properties and those of the bulk solutions. A method is presented to calculate the transport properties of a charged membrane, e.g. enamel, from electromotive force (emf) measurements. These formulas are valid for a system with only a single electrolyte. Corrections for activity coefficients are made. For the calculation of the transport phenomena in charged membranes in more complicated systems a method is presented.

In chapter 3 the instrumental outfit, the preparation of solutions and enamel sections and the method of the measurement of the emf of concentrationcells with enamel membranes is described. The enamel sections used as a membrane in the concentration cells were 200 μm thick slices, sawed from non-erupted bovine incisors. The electrodes used for the emf measurements were silver-silverchloride electrodes of the thermo-electrolytic type.

In chapter 4 the results are presented of electromotive force measurements on bovine dental enamel not treated with a particular chemical agent. The electrolytes which were mostly used are rubidiumchloride and magnesiumchloride. It appears that the membrane potentials calculated with the formulas derived in chapter 2 are in good agreement with the measured emf's.

The results indicate that bovine dental enamel has a negative fixed charge in the RbCl solutions and becomes less negatively or even positively charged in the $MgCl_2$ solutions. This is in agreement with the observations of Waters (Van Dijk et al. 1977) with KCl, NaCl and $CaCl_2$ solutions.

In chapter 5 the results are presented of electromotive force measurements on bovine dental enamel before and after it was treated with a chemical agent. The agents used were orthophosphate, pyrophosphate, trimetaphosphate, hexametaphosphate, phytate, 1,1 ethylhydroxydiphosphonate (EHDP), monofluorophosphate (MFP) and fluoride. All treatments were done with aqueous solutions with a pH of 7. Except for orthophosphate, all agents could increase the ionselectivity of dental enamel with some persistence. The increase varied from 110 to more than 500 percent of its original value. Least successfull were pyrophosphate, trimetaphosphate and fluoride. Very successful was phytate. MFP did increase the ionselectivity much more and with a much greater persistency than either orthophosphate or fluoride.

In chapter 6 a mathematical model for the caries process is presented. This model includes (1) diffusion of ions and molecules, (2) dissolution and recrystallization of mineral and (3) complexation of the various ions present in the system. With computer programs based on the mathematical model it is possible to simulate the development of a subsurface lesion. The most important conditions for a lesion to become a subsurface one appear to be the following : (1) the rates of the dissolution process and of the diffusion processes must be of comparable importance (2) at the pH used the bulk solution must have a buffer capacity greater than that of the phosphate present in the solution and (3) there must be a gradient in either the

solubility product or the dissolution rate constant or the porosity of the enamel. The computer simulations are in quantitative agreement with in-vitro caries experiments presented in the literature. From these experiments and their simulations values were derived for the rate constants of the physicochemical processes during caries.

In chapter 7 a number of in-vitro caries experiments is described. The object of these experiments was to find the minimum set of conditions for obtaining artificially a caries like lesion in dental enamel. The experiments were carried out with 50 mmol $l^{-1}$ acetate buffers of pH = 5.0, which were more or less saturated with synthetic hydroxylapatite. During the experiments the solutions were agitated. We concluded from the experiments that the use of a (charged) gel or the addition of special agents to the buffer is not necessary to obtain subsurface demineralization. Furthermore, these experiments do not support the theories in which the formation of a second solid phase on the crystal surface is supposed to be necessary. Subsurface demineralization occurred if the solutions contained more than about 1 mmol $l^{-1}$ calcium and phosphate.

Finding the main physicochemical factors that determine the caries process and especially the occurrence of the subsurface lesion needs further and thorough investigation,whereby the use of mathematical simulations can be of great help.

Literature Chapter 8.

Van Dijk, J.W.E., Waters, N.E., Borggreven, J.M.P.M. and Driessens, F.C.M. 1977. Some Electrochemical Characteristics of Human Tooth Enamel. Archs. Oral biol 22, 399-403.

König, K.G. 1971. Karies und Kariesprophylaxe. 2nd Ed., 22-95. Goldmann Verlag, München.

Appendices, Introduction.

In the appendices the mathematical aspects of the various computer programs are briefly discussed. The numerical methods used, are chosen either because they were expected to be efficient or because they were already successfully used in other programs. Mostly no research has been done on whether other methods for the same purpose were more efficient, except that it sometimes was tried whether Newton-Raphson (appendix A1) or the method of Powell (appendix A3) was more efficient. The latter often appeared to be less particular in the choice of starting values.

The symbols of appendix G are in general not valid in the appendices. The symbols are mostly chosen conformably to the general usage in mathematical texts. The symbol x for instance is used for an independent variable and f(x) means a function of x. Capitals are used for matrices while the corresponding lower case is used for an element of it. An underscore means that the corresponding variable is a vector or matrix.

## *Appendix A. Numerical methods used in the computer programs.*

### *A.1 Newton-Raphson.*

The method of Newton-Raphson is an iterative method for finding roots of one or a set of non-linear equations. If the set of equations is

$$\begin{aligned} 0 &= f_1(x_1, x_2 \ldots x_n) \qquad 0 = f_n(x_1, x_2 \ldots x_n) \\ 0 &= f_2(x_1, x_2 \ldots x_n) \quad \text{or} \quad 0 = \underline{f}\,(\underline{x}) \end{aligned} \qquad \text{A.1.1}$$

and if $\underline{x}_1$ is an approximation for a vector of roots, then a better approximation is:

$$\underline{x}_{i+1} = \underline{x}_i - (\underline{H})^{-1} \underline{f}(\underline{x}_i) \qquad \text{A.1.2}$$

Here $\underline{H}$ is the n x n matrix of partial derivatives with elements.

$$h_{jk} = \left( \frac{\partial f_j(\underline{x})}{\partial x_k} \right)_{x_{j \neq k}} \qquad \text{A.1.3}$$

If the matrix H is not ill conditioned (Stoer 1972a) and of not to big a size this method can be very efficient if the starting value of $\underline{x}$ is not far from a root of the system. The inversion of $\underline{H}$ is done via Housholder reduction (Stoer 1972b).
The method of Newton-Raphson is used for solving the Donnan equation (chapter 2 and appendix D) as well as for calculating the dependent concentrations from the independent ones in the dynamic caries simulation programs (chapter 6 and appendix F).
A discussion of the method of Newton and Raphson and several improvements and special cases, which we did not use, can be found in Ralston (1965) and Stoer (1972a, 1972b).
The improvements mentioned by these authors are not used because the iterative solution of non-linear equations did, in all programs in which Newton-Raphson was used, cost only a fraction of the total computional effort and thus they would not increase the efficiency of the programs significantly.

*A.2 Gauss Quadrature.*

Gauss quadrature is a simple numerical integration method which approximates the integral of f (x) over the interval

$a \leq x \leq b.$

$$I(f) = \int_a^b f(x)\, dx \qquad \text{A.2.1}$$

by a sum

$$\tilde{I}(f) = \sum_{i=1}^{n} w_i \, f(x_i) \qquad \text{A.2.2}$$

The essential property of the method is that the weights $w_i$ and the absisses $x_i$ are chosen such that the integration error

$$\epsilon = \tilde{I}(f) = I(f) \qquad \text{A.2.3}$$

vanishes if f(x) is a polinomial of degree 2n - 1 or less (Stoer, 1972d).

In the programs TMS3 and TMS4 (chapter 2 and appendix B and D) the Gaussian integration method is used to integrate formula 2.2.9, the activity correction term of the diffusion potential. A four point integration is chosen. The weights are:

$$\begin{aligned} w_1 &= w_4 = 0{,}3478548451 \\ w_2 &= w_3 = 0{,}6521451549 \end{aligned} \qquad \text{A.2.4}$$

If the interval is transformed to the standard interval

$-1 \leqslant x \leqslant 1$, then the absisses are

$$\begin{aligned} x_4 &= -x_1 = 0{,}8611363116 \\ x_3 &= -x_2 = 0{,}3399810436 \end{aligned} \qquad \text{A.2.5}$$

*A.3 Method of Powell 1965.*

The method of Powell (1965) is a method for minimizing a sum of squares of non-linear functions without calculating derivatives. It is especially designed for those least squares problems in which the calculation of derivatives is very costly. The method is in essence a generalized least squares based on finding the solution of the system of equations

$$\left( \frac{\partial}{\partial x_i} f_k(\underline{x}) \right)_{x_{j \neq i}} = 0 \qquad \begin{aligned} i &= 1, 2 \ldots n \\ k &= 1, 2 \ldots m \end{aligned} \qquad \text{A.3.1}$$

for all variables $x_i$ and all functions $f_k$, using a Newton

like iterative procedure (section A.1).

If the sum of squares is

$$Q\ (\underline{x}) = \sum_{k=1}^{m} \{f_k(\underline{x})\}^2 \qquad \text{A.3.2}$$

and a better approximation found by a Newton step is:

$$Q\ (\underline{x} + \underline{\delta}) = \sum_{k=1}^{m} \{f_k(\underline{x} + \underline{\delta})\}^2 \qquad \text{A.3.3}$$

then a still better approximation $Q\ (\underline{x} + \lambda\underline{\delta})$ can be found by minimizing $Q\ (\underline{x} + \underline{\delta})$ along the line with the direction $\underline{\delta}$. The minimum along this line is found by successive quadratic interpolation. The from computional point of view most important property of this method is that the matrix of partial derivatives and its inverse need only to be calculated in the first iteration. For the successive iterations the derivatives are calculated during the quadiatic interpolation. All suggestions concerning the programming of the method made by Powell are followed. Although not suggested by Powell it appeared sometimes usefull to restart the procedure after a number of iterations. The method of Powell is successfully used in the programs TMS3, FLUX and FLUX5 and in a number of smaller programs for calculating equilibrium concentrations of solutions containing calcium, phosphate and one or more weak acids (chapter 2 and 6; appendices C, D and E).

The method is programmed as a procedure which can universally be used in the various programs. Two versions exist one in which the variance-covariance matrix is calculated (formula 2.4.7) and one in which it is not, to save storage and computing time. The procedure is written in PL/1 and consists of some 360 statements.

*Appendix B. The Program TMS4.*

The program TMS4 can be used to calculate the emf of a concentration cell in which a charged membrane separates two solutions of a single electrolyte.
The algorithm for the program TMS4 consists of the following steps:

1 input
2 calculation of the Donnan distribution coefficients for both interfaces using formulas 2.1.10, 2.2.6 and 2.2.10 and Newton-Raphson iteration (appendix A1)
3 calculation of the inner membrane concentrations with formula 2.2.10
4 calculation of the total Donnan potential using formula 2.2.15
5 calculation of the activity correction term of the diffusion potential using formulas 2.2.9 and 2.2.6 and Gaussian quadrature (appendix A2)
6 calculation of the concentration term of the diffusion potential using formula 2.2.4
7 calculation of the total membrane potential using formula 2.2.14
8 output

ad 2
The relative accuracy and the absolute accuracy used as convergence criterion in step 2 was $10^{-6}$ and $10^{-12}$ respectively.
In the example of section 2.2 only nine iterations were required to attain this accuracy.

The combination of the formulas 2.1.10, 2.2.6 and 2.2.10 is used instead of the formula 2.2.13 to enable the correction for the presence of a buffer component. So the summation in formula 2.1.10 is over three components, the kation, the anion and the buffer ion.
The program is written in PL/1 and consists of about 150 statements.

*Appendix C. The Program FLUX.*

The program FLUX calculates the fluxes and the gradients of the concentrations as well as the potential in a membrane for a general electrolyte system.
The problem of finding a general integral for the Nernst-Planck flux equations is transformed to a least squares problem. Both the concentrations and the electrical potential inside the membrane are approximated by a linear combination of Legendre polynomials.
Roughly the program consists of the following steps:

1 input
2 calculation of the values of the Legendre polynomials at the grid points
3 calculation of the Donnan distribution coefficients using 2.1.10, 2.1.11, 2.2.6 and Newton-Raphson iteration (Appendix A1)
4 calculation of the starting values of the coefficients of the polynomials and the fluxes
5 solving the least squares problem by the method of Powell (Appendix A3)
6 output

ad 3

The combination of 2.1.10, 2.1.11 and 2.2.6 gives a polynomial of degree $n = (Z_{max} - Z_{min})$ in which $Z_{max}$ is the charge of the most positive cation and $Z_{min}$ the charge of the most negative anion. The polynomial is solved using the Newton-Raphson method with unity as starting values for the Donnan coefficients.

ad 4

The concentrations are, as a starting point, assumed to be a linear function of the coordinate x. The potential is also assumed to be a linear function of x. As a starting value for the coefficient of the polynomial of the first degree for the potential is used:

$$b_1 = \frac{J_E + \Sigma\, z_i \bar{D}_i\, a_{i1}}{\Sigma\, z_i^2 \bar{D}_i} \qquad \text{C.1.4}$$

where $a_{i1}$ is the slope of the concentration of compound i which was supposed to be a linear function of x and $J_E$ is the electrical current density. This formula is a rearrangement of 2.1.4 substituted in 2.3.3 with $\frac{dC_i}{dx} = a_{i,1}$ and $\frac{dE}{dx} = b_1$.
The starting values for the fluxes are obtained from 2.1.4 using the starting values for $\frac{dC_i}{dx}$ and $\frac{dE}{dx}$.

ad 5

For each iterative step of the procedure of Powell the following calculations must be done

1 calculation of the concentrations from the coefficients of the Legendre polynomials
2 calculation of the derivatives of the concentrations and of the electrical potential from the coefficients of the Legendre polynomials

3 calculation of the derivatives of the logarithms of the activity coefficients from the concentrations and their derivatives

4 calculation of the function values of the formulas 2.3.1, 2.3.2 and 2.3.3

5 calculation of the sum of the squares of these function values

The relative and absolute accuracy required for convergence were $10^{-6}$ and $10^{-12}$ respectively. The convergence criterion must be fulfilled for the conditions 2.3.1, 2.3.2 and 2.3.3 for the coefficients of the polynomials and for the fluxes. The number of iterations necessary for solving the example of Schlögl (section 2.3) was sixteen.
The program is written in PL/1. Not including the procedure of Powell the program consists of about 300 statements.
Of this program more elaborate versions have been made to include the equilibria of the oral cavity such as those of orhtophosphoric, carbonic, acetic and lactic acid as well as the various calcium complexes. It appeared that for these complex systems it is very useful to scale all variables so that most of their starting values are unity.

*Appendix D. The Program TMS3.*

The program TMS3 is used to calculate from emf measurements the fixed charge of a membrane and the ratio of the diffusion coefficient of the cation and anion of a single electrolyte.
The program TMS3 is designed to calculate, from emf measurements, the concentration of the fixed charge of a membrane and the ratio of the diffusion coefficients of the cation and anion of

a single electrolyte.

The program consists roughly of the following steps:

1 input

2 calculation of starting values for $w\bar{X}$ and $\bar{D}_+/\bar{D}_-$

3 calculation of the minimum of the sum of squares of the difference between the measured and the calculated emf's $(\sum_i (E_i - \hat{E}_i)^2 \quad i = 1, 2...n)$

4 output

ad 2

At the start of the calculations $w\bar{X}$ is supposed to be zero. The starting value for $\bar{D}_+/\bar{D}_-$ is calculated with formula 2.2.12. When the activity factors and the Donnan coefficients are assumed to be unity, 2.2.12 becomes

$$\bar{E}_C = -\frac{RT}{F} \frac{\frac{\bar{D}_+}{\bar{D}_-} - 1}{Z_+ \frac{\bar{D}_+}{\bar{D}_-} - Z_-} \ln \frac{C''}{C'} \qquad \text{D.1.1}$$

and thus

$$\left(\frac{\bar{D}_+}{\bar{D}_-}\right)_{start} = \frac{Z_+ \bar{E}_c + \frac{RT}{F} \ln \frac{C''}{C'}}{Z_- \bar{E}_c + \frac{RT}{F} \ln \frac{C''}{C'}} \qquad \text{D.1.2}$$

(for symbols see appendix G)

ad 3

The minimum of the sum of squares is calculated using the method of Powell (1965) (see appendix A3). For the calculation of the sum of squares for each step of the iterative procedure the emf's have to be calculated from the current values of $w\bar{X}$ and

$\bar{D}_+/\bar{D}_-$ and the concentrations used. This is done using the same algorithm as in the program TMS4 (except of course step 1) (in fact the programs TMS3 and TMS4 make use of the same subprograms for this calculation). The calculation of the example of section 2.3 costs five iterations of the Powell procedure. The program is written in PL/1 and consists of about 200 statements excluding the Powell procedure.

*Appendix E. The Program FLUX5.*

The program FLUX5 is designed to calculate the quasi steady state of the system in which (in vitro) caries develops. The program FLUX5 is essentially the same as the program FLUX (appendix C and section 2.3). The only important difference is that the conditions 6.3.1 replaces 2.3.1 so that the flux of each component needs not to be constant throughout the entire thickness of the enamel membrane. Condition 6.3.1 in combination with formula 6.3.2 becomes

$$\frac{d\bar{C}_{ij}}{dt} = -\frac{1}{\alpha_j}\frac{dJ_{ij}}{dx} + \nu_i\, v_{dis\ j} = 0 \qquad \text{E.1}$$

The main program consists of the same six steps as FLUX. The intermediate steps, which have to be carried out for each iteration of the Powell procedure (step 5 appendix C) are a little more extensive. In the first intermediate step the dependent concentrations are calculated from the independent ones using the equilibrium conditions (section 6.2) and the rate of dissolution is calculated using one of the formulas 6.2.2 or 6.2.3. After the calculation of the derivatives of the concentrations and electrical potential (step 2) and of the logarithms of the

activity coefficients (step 3), the fluxes are calculated as well as their derivatives (formulas 2.2.1 and 6.2.1). The function values are calculated using the formulas E.1, 2.3.2 and 2.3.3. After the excecution of the Powell procedure the rate of demineralization is calculated by numerical integration (formula 6.2.4). The rest of the program is again the same as the program FLUX (appendix C).

*Appendix F. The Program CASIM.*

The dynamic caries simulation program CASIM is a program based on the Continuous Modeling Program (CSMP) (IBM 1975). The user of CSMP has to supply a description of the system to be simulated in terms of for instance differential equations. This description, which is partially in CSMP language elements and partially in FORTRAN is, by the CSMP translator, transformed into a FORTRAN subroutine. This subroutine, which is named UPDATE, is called by the CSMP main program which contains all other routines necessary for a continuous simulation program like integration procedures for solving the differential equations of the model.

In our model the enamel is divided into eleven compartments. For each compartment and for each of the five independent components a differential equation of the type 6.3.2 is formulated. This results in a system of 55 differential equations for the concentrations. Further the amount of mineral dissolved in each compartment is described by the differential equation of formula 6.2.2 or 6.2.3. The attractiveness of using a program like CSMP for solving the simulation problem with over sixty complicated differential equations is that these equations are solved "simultaneously" and thus in fact simulates an analogue computer.

This is very important because the outcome of the integration of one out of the set of differential equations changes all other differential equations. And thus the outcome of a procedure in which the equations were integrated one by one would certainly not be correct.

The concentrations of all components in each of the eleven compartments are calculated from the total concentrations of calcium, phosphate, organic acid and of $H^+$ ions bound to phosphate and organic acid or free in solution, by means of the equilibrium conditions (formula 6.2.5). Because of the calcium phosphate and calcium acetate or lactate complexes, the system of equations that results from the equilibrium conditions, is a nonlinear one. By the introduction of activity coefficients this system becomes relatively unstable. This can be avoided by introducing an approximation to the ionic strength.
The ionic strength is:

$$I = \tfrac{1}{2}\{[Na^+] + 4[Ca^{++}] + [H^+] + [H_2PO_4^-] + 4[HPO_4^{=}] + [Z^-] + [CaH_2PO_4^+] + [CaZ^+] + [Cl^-] + [OH^-]\} \qquad F.1$$

Note that the concentration of $PO_4^{\equiv}$ is neglected.
The electroneutrality equation is:

$$0 = \tfrac{1}{2}\{[Na^+] + 2[Ca^{++}] + [H^+] - [H_2PO_4^-] - 2[HPO_4^{=}] - [Z^-] + [CaH_2PO_4^+] + [CaZ^+] + [Cl^-] - [OH^-]\} \qquad F.2$$

F.1 and F.2 together yield:

$$I = [Na^+] + 3[Ca^{++}] + [H^+] + 2[HPO_4^{=}] + [CaH_2PO_4^+] + [CaZ^+] \qquad F.3$$

If we approximate the three calcium terms by three times the total calcium concentration and neglect the $HPO_4^{=}$ and $H^+$ concen-

tration we obtain the approximated ionic strength

$$\tilde{I} = [Na^+] + 3 \cdot [Ca_{tot}] \qquad F.4$$

Activity coefficients calculated with F.4 did in no simulation differ more than a few percent from those calculated with formula F.1. The system of non-linear equations can be solved by Newton Raphson iteration (Appendix A1). If, however, the $Ca^{++}$ concentration is supposed to be equal to the total calcium concentration then all concentrations can be calculated directly, which makes the execution of the program a factor three faster. This assumption in fact means that the complexes are formed with some delay. The outcomes of a simulation with the iterative method or the direct one differ one or two percent only.

All derivatives necessary to calculate the fluxes and the rates of change of the total concentrations (formula 6.3.2) are obtained by numerical differentiation (second degree interpolation).

The complete program is rather large. If 512 kilobytes of core memory is used, the simulation of a few hours caries attack costs about ten minutes time of the central processore unit of the IBM 370/158.

## *Appendix G. Glossery.*

### *G.1 List of symbols for physical quantities.*

a Electrochemical activity.

A Parameter in Debye Hückel and related formulas.

b Correction factor in Guggengeim's formula for activity coefficients.

C Concentration.

$d$ Thickness of a diffusion film.

$d_1$ Depth in carious enamel at which the demineralization is most severe.

$D$ Diffusion coefficients. (units of membrane surface per unit of time)

$E$ Electromotive force (e.m.f.).

$E_A$ Activity correction term of the diffusion potential.

$E_C$ Concentration term of the diffusion potential.

$E_D$ Donnan potential.

$E_E$ Electrode potential.

$E_M$ Total membrane potential.

$E_O$ Standard potential of an electrode.

$f$ Activity coefficient.

$F$ Faradays constant.

$\Delta F$ Free energy of activation (section 2.1 only).

$h$ Constant of Planck (section 2.1 only).

$H$ Variance covariance matrix (lower case for elements of the matrix).

$I$ Ionic strength.

$I_A$ Ion product of a solution for hydroxylapatite.

$I_D$ Ion product of a solution for DCPD

$J$ Flux of ions and molecules.

$J_E$ Current density.

$K$ Friction coefficient.

$k_{dis}$ Dissolution rate constant.

$K$ Rate constant in the theory of absolute reaction rates (section 2.1 only).

$K_A$ Solubility product of apatite.

$K_W$ Dissociation constant of water.

$K_1$, $K_2$, $K_3$ The three dissociation constants of orthophosphoric acid.

$K_4$ Dissociation constant of acetic acid or lactic acid.

$K_5$ Dissociation constant of the calcium complex with primary phosphate.

$K_6$ Dissociation constant of the calcium complex with secondary phosphate.

$K_7$ Dissociation constant of the calcium complex with acetate or lactate.

L Phenomenological coefficient.

N Avogadro's number.

Q Sum of squares in a least squares problem.

r Donnan distribution coefficient.

R Gas constant.

s Calculated standard error (subscript refers to the quantity on which it is applicable).

S Selectivity for a 1,1-electrolyte (subscript denotes the concentration at which it is valid in mol $l^{-1}$).

t Time.

T Absolute temperature.

$v_{dem}$ Rate of demineralization.

$v_{dis}$ Rate of dissolution.

x Coordinate perpendicular to the membrane (enamel) surface.

X Concentration of the fixed charge.

Z Charge of a component including its sign.

$\alpha$ Effective fractional volume of the transport phase.

$\Gamma$ Matrix of partial derivatives (lower case for the elements of the matrix).

$\delta$ Thickness of the membrane (enamel).

$\lambda$ Jump distance in the theory of absolute reation rates.

$\mu$ Chemical potential.

$\mu_e$ Electrochemical potential.

$\nu$ Number of ions per molecule.

$\rho$ Ratio of activity coefficients of the bulk solution to the pore solution at the interface.

$\chi$ Driving force.

$\omega$ Sign of the fixed charge.

*G.2 List of subscripts, superscripts and special symbols.*

i Index denoting a component or number in series (section 2.4 only).

j Index denoting a place.

k Index denoting a component.

+ Subscript denoting a cation.

- Subscript denoting an anion.

$\pm$ Subscript denoting a mean value for cation and anion.

$^-$ Superscript denoting a quantity in the transport phase (bar).

$'$ Superscript denoting a quantity in the left bulk phase or interface (quote).

$''$ Superscript denoting a quantity in the right bulk phase or interface (double quote).

$\hat{}$ Superscript denoting an expected value (in contrast to a measured value).

$^*$ Superscript denoting an apparent quantity

$\sim$ Superscript denoting a quantity calculated with an approximating formula.

$[A]$ Denotes the concentration of the component A (square brackets).

p Prescript denoting the negative logarithm of an activity, ionproduct or solubility product.

*G.3 List of mathematical symbols and operators.*

| | |
|---|---|
| d | Differential operator. |
| ∂ | Partial differential operator. |
| e | Base of the natural logarithm. |
| ln | Natural logarithm. |
| log | Common logarithm. |
| T | As a superscript denotes the transpose of a matrix. |
| Σ | Summation operator. |

*G.4 Names and formulas of some chemicals.*

| | |
|---|---|
| chlorhexidine | N,N"-Bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazotetradecane diimidamide $C_{22}H_{30}Cl_2N_{10}$ |
| DCPD | Brushite, calciummonohydrogen phosphate dihydrate, dicalcium phosphate dihydrate $CaHPO_4 \cdot 2H_2O$ |
| EHDP | 1,1 ethylhydroxydiphosphonic acid $C_2H_8O_7P_2$ |
| Glycerol | 1, 2, 3 propanetriol $C_3H_8O_3$ |
| HEC | Hydroxyethylcellulose $(C_{12}H_{19}O_8)_n$ |
| HEPES | 2[ 4(2-hydroxyethylpiperazinyl-(c)-]-ethanesulfonic acid, N-2-hydroxyethylpiperazine-N'-2-sulfoniacid $C_8H_{18}N_2O_4S$ |
| MFP | monofluorophosphate $FPO_3^=$ |
| OHA | Hydroxylapatite $Ca_{10}(PO_4)_6(OH)_2$ |
| Phyticacid | myo-inositol-hexakis (dihydrogenphosphate), inositolhexaphosphoric acid $C_6H_{18}O_{24}P_6$ |
| Sorbitol | D-glucitol $C_6H_{14}O_6$ |

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## Curriculum vitae.

Ik ben op 10 maart 1946 geboren in Almelo, alwaar ik de lagere school en de afdeling HBS-B van het Erasmuslyceum doorlopen heb. In 1963 ben ik scheikunde gaan studeren aan de Rijksuniversiteit te Leiden, alwaar ik in 1968 het kandidaatsexamen (letter G) behaalde. Het doktoraal examen met als hoofdvak organische scheikunde en als bijvakken numerieke wiskunde en theoretische organische scheikunde behaalde ik eind 1971. Na het vervullen van de dienstplicht bij de Koninklijke Marine trad ik in 1973 in dienst bij de Katholieke Universiteit te Nijmegen, alwaar dit proefschrift werd voorbereid. Thans ben ik als scheikundige verbonden aan de Werkgroep Tand- en Mondziekten van de Gezondheidsorganisatie T.N.O. te Utrecht.

# STELLINGEN

## I

De snelheid van de diffusieprocessen door de porië̈n in het tandglazuur en de snelheid van het oplosproces van het mineraal zijn van vergelijkbaar belang voor de snelheid van het kariësproces.

## II

Het onderzoek van Moreno en Zahradnik toont niet aan dat de vorming van een tweede vaste fase (zoals brushiet) op het glazuurapatiet in het algemeen noodzakelijk is voor het behoud van de oppervlaktelaag van het glazuur tijdens kariës. J.Dent.Res. 53 , 226-235(1974).

## III

De ruimtelijke ordening van de kristallieten in het tandglazuur is niet een noodzakelijke factor voor de verklaring van het ontstaan van een onderhuidse ontkalking.

## IV

Het is op grond van theoretische beschouwingen niet te verwachten dat een compleet herstel van een onderhuidse ontkalking van een tand door applicatie van speciale agentia, zal kunnen geschieden binnen een voor de algemene tandheelkundige praktijk aanvaardbare tijdsduur.

## V

Fundamenteel wetenschappelijk onderzoek op het gebied van de tand- en mondziekten is onontbeerlijk voor het toegepast en klinisch onderzoek op dat terrein.

## VI

Het gebruik van wiskundige modellen van een systeem kan het laboratorium en dierexperimenteel onderzoek aan dat systeem veel doelgerichter en efficiënter maken.

VII

Het gebruik van Algol, of van talen waarvan Algol een onderdeel vormt, verdient voor de publikatie van algorithmen en voor onderwijsdoeleinden de voorkeur boven andere gebruikelijke programmeertalen.

VIII

Het verdient aanbeveling dat kinderartsen speciale aandacht schenken aan de voorlichting van huisarts en ouders indien een zuigeling behoort tot een groep met een verhoogd risico voor wiegedood.

IX

De arts die betrokken is bij een mogelijk geval van wiegedood zal bij het adviseren van de ouders omtrent het laten plegen van obductie niet alleen "het nare idee van het moment" doch ook dat van "de zekerheid op lange termijn" moeten overwegen. Bovendien mag hij het medisch wetenschappelijk belang niet uit het oog verliezen.

X

Het aanbrengen van een voorziening in auto.s die voorkomt dat de verlichting ongewenst blijft branden na het verlaten van de auto, zal de automobilist eerder doen besluiten zijn verlichting te ontsteken bij verminderd zicht en zal daardoor de verkeersveiligheid wezenlijk bevorderen.

J.W.E. van Dijk 7 april 1978